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THE RENAL LESION IN OBSTRUCTIVE JAUNDICE.

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"Cholemic nephrosis" has received little attention from those interested in the problems of bile tract disease even though reports in the literature suggest that renal injury secondary to hepatic disease or injury may be more frequent than is generally believed. We have attempted to determine the importance of the renal injury in a group of patients with obstructive jaundice in the surgical wards of this hospital and in the experimental animal following common duct obstruction and cholecystectomy.

Wilensky,[26b] in a recent review of the subject of "liver death," points out that the hepatorenal syndrome, in addition to its incidence in liver and biliary disease, has been seen to follow operations for other conditions such as carcinoma of breast, gastric ulcer and so on; following the use of certain drugs; in the toxemia of pregnancy, thyrotoxicosis, burns and intestinal obstruction. Wilensky stresses the phylogenetic relation between the liver and kidney, which in certain mammals does not involute, leaving a portal vein to the kidney. Anastomoses between the vessels to the liver and kidney are at times observed in both animal and man.

Elsom,[6] studying the renal function of 16 patients with obstructive jaundice and 1 with arsenical hepatitis with the Addis method,

found an increase in casts, epithelial cells and white blood cells, and in erythrocytes in 8 of the patients, and an increase in albumin in only 4 patients. Elsom found that the absence of albuminuria is a differential diagnostic point between "cholemic nephrosis" and other forms of nephrosis. Blood urea nitrogen determinations ranged from 15.5 to 28.1 mg. % in 8 patients and the urea clearance ranged from 80% of normal to a significant reduction in half the cases.

The occurrence of uremia in hepatic disorders was reported as early as 1876,[24] although at that time renal involvement was thought to be absent. Helwig and Schutz[11a] reported severe renal damage with fatal uremia in 2 cases of liver disease (1 neoplastic, the other associated with severe trauma of the liver). They also reported 4 cases of gall bladder disease with evidence of renal damage. They believed that the damage was not due to an increase in the bile salts in the blood since only 2 of the patients were jaundiced. The increase in bile salts in the blood after obstructive jaundice lasts for only a few days while the nephrosis persists during the period of jaundice. Bartlett[2] found 6 patients of 56 consecutive cases with bile tract infection who had renal damage with oliguria. Not all of these were jaundiced. Meyers and co-workers,[16] in a study of 21 cases of jaundice, found 7 with high blood urea nitrogen figures. Eiss,[5] Schutz and associates[19] and Rowntree[18] have noted uremia in patients following biliary surgery. Others[7 10 15 22 23] have noted evidence of renal injury ranging from albuminuria to uremia and death in disease of, and trauma to, the liver.

Wilensky,[26a] in a study of the relation of nitrogen retention to liver and biliary tract disease, found that the blood urea nitrogen was elevated: (1) in 67% of mild grades of biliary tract disease without complications; (2) in 86% of mild or moderately severe disease with jaundice; and (3) in all but 1 case of mild grades with jaundice and nephritis.

The mechanism involved in renal damage following common duct obstruction or liver injury is not clearly understood. Lichtman and Sohval[13] believe that the anuria and azotemia are due to a prerenal deviation of fluids. They feel that this is the result of disturbances of those liver functions which are concerned with water and mineral balance and water is not available for renal excretion. It is their opinion that operation, jaundice, shock, decompression and vomiting are not essential in producing renal lesions and that use of the term "hepatorenal" syndrome should be avoided. Stewart and Cantarow,[21] following the intravenous injection of dogs with sodium dehydrocholate, an unnatural bile salt, were able to produce severe renal lesions demonstrating regressive and regenerative changes in the tubules.

In common duct obstruction there is usually some hepatocellular damage and it is not unlikely that other substances toxic to the kidney may be released from the injured liver cells. Ravdin[17]

found an increase in histamine and choline in livers of dogs in which the common duct had been ligated over a period of days.

Boyce and McFetridge,[3] following a clinical and experimental investigation of the mechanism of so-called "liver death," came to the conclusion that the hepatorenal syndrome is due to the release of the common duct obstruction. This, they believe, permitted the escape of a toxin which, acting upon an overtaxed kidney, causes a breakdown of the convoluted tubules. This thesis is also held by others.[11b, 19]

Heyd[12a, b] is of the opinion that the kidney lesions and the anuria seen in liver disease are unrelated to the liver. He divides "liver deaths" into three groups: 1, hepatic exhaustion due to surgical intervention; 2, hepatic failure following release of the obstruction in jaundiced patients which causes a reactive hyperemia in an already compromised liver; 3, pancreatic intoxication in disease of the common duct and pancreatitis without jaundice. Dourmashkin[4] presented an interesting report on 2 patients with coëxisting hepatic cirrhosis and urethral stricture, who, following urethral instrumentation, developed an acute hepatorenal syndrome and died. Their urine showed albumin and red cells and the one patient at autopsy showed epithelial degeneration of the kidney. Another patient, assumed to have cirrhosis, developed a mild icterus following intra-ureteral manipulation for a stone and recovered. In a review of 500 deaths following operations on the gall bladder and bile ducts, Stanton[20] reported that 24 were due to renal failure.

We feel that the renal lesion in common bile duct obstruction and in instances of more severe liver damage should not be considered as a minor complication, but as a major one. It is true that in most instances permanent injury does not occur if relief of the aggravating condition is afforded promptly, but there is a not inconsequential group which goes on to anuria and a uremic death.

Pathologic Anatomy. The pathologic change of the kidney of "cholemia nephrosis" has been described[25] as being confined mainly to the tubules. These exhibit dilatation with a narrowing and degeneration of the epithelial cells. There is occasionally partial obstruction of the tubules, which contain much granular débris and occasional casts. Bile pigment may be seen in the epithelial cells and in the intratubular spaces. There are often localized areas of fibrosis and edema in the interstitial tissue. In other instances one finds sclerotic patches with hyalinized glomeruli and atrophic tubules. As a rule the glomeruli are normal, but may show an increase in, and some swelling of, the endothelial cells. Granular débris is often present in Bowman's capsule and there may be irregular thickening of the membrane.

Lieber and Stewart[14] classified microscopic renal changes in patients with bile duct obstruction in their material as follows: (1) In complete and permanent obstruction of the common duct

due to primary carcinoma of the pancreas a few regressive changes and much green, yellow and black pigment, although there is not much bilirubin; (2) in carcinoma of the pancreas with death following surgical decompression, regressive changes are prominent, although there is diminution of renal pigment; (3) in obstructive jaundice due to calculi with death following decompression, regressive changes more marked than in (2); (4) in calculous disease without jaundice with death following surgical procedures, damage is less than in (2) and (3).

Clinical Studies. In order to investigate the incidence of renal injury following bile duct obstruction the histories of 32 patients with obstructive jaundice on Surgical Divisions "B" and "E" were studied. Most of these were cases of calculous obstruction, although 3 had pancreatic lesions, 2 stricture of the common duct, and 1 a carcinoma of the ampulla of Vater. All were jaundiced for periods ranging from 3 days to 9 months. All patients came to operation and 5 died.

Six of the 32 excreted urine with a specific gravity of 1.015 or less, and 16 excreted urine with a specific gravity of 1.020 or less. At least a trace of albumin was present in the urine in every case; it was present in a moderate amount or more in 17. This is not in accord with Elsom's[6] statement that absence of albuminuria is a distinctive feature of "cholemic nephrosis." Casts were seen in the urine of 13 patients, although not in large numbers except in 5. Red blood cells in abnormal amounts appeared sporadically in 4 cases; white cells were noted in pathologic quantities in 17 cases. As these patients were not always studied from a renal viewpoint, blood urea nitrogen, urea clearance and phenolsulphonephthalein excretion determinations were few. One of those that died had a rising blood urea nitrogen, values of 43 mg.% 4 days before and 54 mg.% 2 days before death. The recorded causes of death of the other 4 were "liver shock," myocardial failure and hemorrhage, but a review of the records is not convincing that these were the causes of death. There was no notable difference in the urinary findings before and after operation.

In order to attack the problem from another angle, a study was made of cases dying of hepatic or biliary tract disease. Included in this study are all the patients who died between 1922 and present date of hepatic or biliary disease on the services of Dr. Müller and Dr. Ravdin, a total of 61 cases. Nearly all of these cases are known definitely to have been jaundiced. Operations were performed on 50 patients; autopsies, with complete study of the kidney, on 20. Chronic calculous cholecystitis and common duct obstruction was the diagnosis in 28 cases; the next most common condition was carcinoma of the head of the pancreas (9 cases); there were 6 cases of non-calculous common duct obstruction; 7 of cirrhosis of the liver; 2 of carcinoma of the gall bladder; and 1 case each of traumatic

rupture of the liver, acute hepatitis, carcinoma of the common duct, liver degeneration and necrosis of unknown etiology, carcinoma of transverse colon, retroperitoneal sarcoma, carcinoma of the liver (unverified), hepatic failure with septicemia and carcinoma of the stomach.

Low specific gravity of the urine was found in approximately the same percentage as in the first group. Of the 61 patients, 13 had a consistent specific gravity of 1.015 or less, and 30 had a specific gravity of 1.020 or less. Albumin, a trace or more, was seen in 55 cases; 27 cases showed a moderate or greater amount. This strengthens our impression that absence of albuminuria is not a distinguishing character of the renal changes associated with biliary or hepatic dysfunction.

TABLE 1.—INCIDENCE OF URINARY FINDINGS IN THE CLINICAL CASES STUDIED.

Series.	No. of patients.	Specific gravity.		Albumin.		Casts.		R.B.C.		W.B.C.		B.U.N.
		Below 1.015.	Below 1.020.	Pres.	Mod. or more.	Pres.	Many.	Pres.	Many.	Pres.	Many.	40–159 mg.%.
I	32	6	16	32	17	13	5	6	4	17	10	
II	61	13	30	55	27	44	27	24	8	57	21	10

Casts were seen in the urine of 44 patients; of these, 27 showed casts regularly and in large amounts. Twenty-four patients showed some red blood cells in the urine, and 8 of these had erythrocytes in large amounts. As in the first group of patients studied, there was an insufficiency of tests of renal function. However, on 11 patients upon whom phenolsulphonephthalein tests were made, the excretion was normal. The blood urea nitrogen was high in 10 patients, ranging from 40 to 159 mg.%. In 18 others, upon whom the determination was made, the range was between 8 and 27 mg.%. Although the clinical diagnosis of uremia was considered in only 4 of these patients, we cannot help but feel that the renal injury was a factor in the morbidity and mortality of more than 4 cases.

In practically all instances in which patients were discharged as improved, the excretion of albumin and casts cleared up before the patient left the hospital. The same is true of the excretion of red and white blood cells. In the series of patients who died, however, the urinary abnormalities continued until death (Table 2). Tubular degeneration was seen in all but 2 cases. In nearly half the cases (9) there was fibrosis or hyalinization of glomeruli. Localized areas of round cell infiltration were noted in 4 cases, 2 of these being in proximity to damaged glomeruli. Thirteen cases exhibited sclerotic changes in the small and large blood-vessels of the kidney.

Experimental Studies. We have investigated the pathologic changes in the kidney following experimental obstruction of the

common duct of 6 dogs. Using ether anesthesia, the common duct of each of these animals was doubly ligated and divided. A biopsy was taken from one kidney for control purposes.

So far 3 dogs have died during observations (Table 3) and 2 have been sacrificed. The lengths of life postoperatively were 22, 44, 42, 68 and 97 days. It is interesting to note that 4 of these 5 had perforated duodenal ulcers. Urinalyses and tests of phenolsulphonephthalein excretion were made at intervals. Some authors have reported a rise in phenolsulphonephthalein excretion in biliary obstruction while others have found no change.[1,9]

TABLE 2.—RENAL CHANGES IN THE 20 CASES IN SERIES II THAT CAME TO AUTOPSY.

Patient.	Tubular change.	Glomerular change.	Interstitial nephritis.	Blood-vessels.
A. R.	None	Hyalinization; thickened capsules	Subcortical	
A. C.	Cloudy, swelling	Fibrosis	Periglomer.	Fibrosis—retention cysts
M. R.	Epith. irreg., granular cells swollen	None	None	Engorged
S. B.	Swollen, granular, desquam.	None	None	Large arteries and arterioles thickened
J. S.	Swollen, granular	Number reduced	None	Few fibrosis scars
N. A.	Swollen, granular	None	None	Sclerotic (many cysts with and without hemorrhage)
I. K.	Granular	Hyalinization, fibrosis	None	Intimal thickening
E. K.	Flat, poorly preserved	Contain amorph. ppt.	None	None
F. H.	Vacuolated; am. masses of yellowish pigment	None	None	None
J. McC.	Marked cloudy swelling	None	None	Engorged
J. B.	Degen., desquam.	Thickened, gran., cap. loops in several	None	Large arteries, thickened
A. G.	Irreg. epithelium	Contain pink-staining material		
P. S.	Swollen, granular	Shrunken, fibrosis	None	Thickening of afferent arterioles; intimal change in small arteries
N. M.	Sl. cloudy swelling	None	None	Rare mesial arteriolar thickening
N. F.	Sl. degeneration	Mod. hyalinization	None	Some hyalinization of arterioles
J U.	Swollen, irreg., granular	Occ. hyalinized	None	None
J. C.	Irreg., fat change, swollen	Hyalinization; areas of interst. nephritis	Occ. sub. capsular	Thickening, hyalinization of small arterioles
O. S.	None	Obliterated in places by wedge-shaped scars	Infilt. about glomeruli	Medial sclerosis of large vessels
R. P.	Swollen, granular; hyaline change	Fibrosis	None	Thickened with hyalinization in small arterioles (few)
R. McN.	Necrotic, disintegrating	Less cellular than normal; occ. scar glomerulus	None	Arterioles mod. sclerotic.

The 6 dogs all showed albumin in the urine after operation; all but 1 showed white blood cells and only 2 showed red blood cells in the urine. Casts were absent in all cases. Bile-stained renal cells, a condition thought by some[8] to be indicative of renal damage in obstructive jaundice, were noted in the urine of 3 animals. Thus the urinary picture of these animals was not as severe as in the clinical cases.

The method of determining the phenolsulphonephthalein excretion was as follows: The animals were catheterized and the bladder emptied. One cubic centimeter of phenolsulphonephthalein (6 mg.

TABLE 3.—PHENOLSULPHONEPHTHALEIN EXCRETION OF DOGS FOLLOWING EXPERIMENTAL OBSTRUCTION OF THE COMMON DUCT.

Dog 277.		Dog 341.		Dog 380.		Dog 496.		Dog 644.	
Date.	PSP, %.	Date.	PSP, %.	Date.	PSP, %.	Date.	PSP, %.	Date.	PSP, %
1–6	Operation	1–13	Operation	1–25	90	2–15	55	3–18	55
1–25	70	2–6	60	1–27	Operation	2–17	60	3–20	55
2–10	55	2–20	70	2–8	70	2–17	Operation	3–22	Operation
3–8	75	2–24	Death	3–8	75	3–8	55	4–1	60
3–15	70	..	...	3–9	55	3–15	45	4–5	70
3–16	70	..	...	3–10	70	3–17	35	..	Death
3–17	70	..	...	3–15	60	3–20	60		
3–21	70	..	...	3–17	55	3–21	60		
4–3	70	..	...	3–20	60	3–22	60		
4–6	55	..	...	3–21	45	3–24	65		
4–8	55	..	...	3–22	35	4–3	60		
4–14	Death	..	...	3–24	70	4–6	60		
				3–27	60	..	Sacrificed		
				4–1	70				
				4–5	Sacrificed				

per cc.) was injected intravenously. Tap water (500 cc.) was given by stomach tube. At the end of an hour, the bladder was emptied (the catheter having been kept in place) and washed out with 60 cc. of tap water. The percentage of dye excretion was determined by comparison with a Dunning colorimeter (Table 3). It can be seen that there were no consistently striking depressions of the phenolsulphonephthalein elimination, although in 2 animals there was a depression with return to normal.

In 2 dogs (380 and 496) the phenolsulphonephthalein excreted was but 35%. In Dog 644 there is a slight rise in the phenolsulphonephthalein excretion following operation.

Pathologic Findings. In the sections of kidney obtained at autopsy there was no significant change found in the glomeruli. The blood-vessels and interstitial tissue were also normal. The main change was in the tubules, which were dilated and which showed compression of the lining epithelium. There were areas of vacuolization, fragmentation and desquamation in the tubular epithelium. Occasional casts were seen in the tubules; in the cells, in some instances, was seen a deposition of yellowish-brown pigment. These changes are essentially the same as those previously described in human "cholemic nephrosis."

Summary. Our clinical and experimental studies strongly confirm Elsom's[6] observations on renal changes in hepatic disease, except that our patients had a high incidence of albuminuria and a number of high blood urea nitrogen figures were noted.

A preëxisting renal lesion is intensified by a secondary "cholemic nephrosis." The high incidence of glomerular changes in the

patients that died suggests that a preëxisting renal lesion may have been present. This is strongly suggested by the fact that these changes predominated in the elderly patients.

If no previous renal injury exists, the evidences of "cholemic nephrosis" disappear rapidly following release of the obstruction. The major injury in this disorder is confined to the tubules.

The nephrosis associated with bile duct obstruction may be a determining factor in the final outcome and should not be looked upon as an inconsequential complication.

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TUBERCULOSIS OF THE NASOPHARYNX.

POSITIVE SPUTUM WITH A ROENTGENOGRAPHICALLY NEGATIVE CHEST.

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SINCE Koch's[4] classic demonstration of tubercle bacilli in the sputum and Munier's[7] demonstration of their presence in the gastric contents, either finding has been considered indicative of a tuberculous lesion in the pulmonary parenchyma. During more recent years, attention has been called to the fact that tubercle bacilli found in the sputum may arise from lesions of the tracheobronchial tree. In the past 2 years, we have had occasion to observe 2 cases which suggest to us that still another source of tubercle bacilli in

the sputum should receive more serious clinical study. Both of these had tuberculous lesions of the nasopharynx; both raised their sputum in the characteristic manner for postnasal discharge; in both the sputum contained readily demonstrable tubercle bacilli; and in one, frequent roentgenograms of the chest and a bronchoscopic examination were negative for evidence of tuberculosis.

The following reports in the literature emphasize the need for further separation of the several possible sources of tubercle bacilli in the sputum and gastric contents when a pulmonary lesion cannot be demonstrated.

Steidl and Heise,[15] in 1931, in discussing the value of old tuberculin administered subcutaneously for diagnosis in 419 cases of "suspected pulmonary tuberculosis," found that 35 had a history of tubercle bacilli in their sputum before admission to Trudeau Sanatorium or that tubercle bacilli were found during their residency there. Later in 1934, Sampson and Brown,[13] reporting from the same place, state, "Another study of 312 patients without definite roentgenological change presenting one or more of the other four cardinal diagnostic criteria, revealed that 59 were said to have had tubercle bacilli in the sputum at some time, often before admission. . . ." In a recent personal communication with one of the authors[12] it is now roughly estimated that this number approaches the 100 mark. Some of these cases later developed roentgenographic signs of pulmonary tuberculosis; in others, giving a history of having had tubercle bacilli in their sputum, this may have been an erroneous observation; in still others, the focus of tuberculosis in the respiratory tract may have been in the nasopharynx, the trachea, or the bronchi.

Meersseman,[6] in June of 1938, reported from France a group of people he called "healthy expectorators" of tubercle bacilli. He apparently became interested in this problem in 1930 when he discovered a medical student and a young soldier with tubercle bacilli in their sputum and with negative chest *x*-ray findings. These 2 patients were not proved to be carriers of tubercle bacilli by guinea pig inoculation but the morphology and staining were so typical that the author did not doubt their being tubercle bacilli. Later, among 84 healthy medical students who were radiographically negative, he found one whose sputum produced a tuberculous death in a guinea pig 4 months after inoculation. In 1934, he examined 51 medical students with negative chest *x*-rays and 69 soldiers with negative fluoroscopic examinations. The sputum of each of these students and soldiers was inoculated into guinea pigs. Two medical students with negative chest *x*-rays had tubercle bacilli in the sputum and no *x*-ray changes developed over a 3-year period of observation. A third, a soldier, was found to have tubercle bacilli in the sputum by guinea pig inoculation and the chest *x*-ray taken after this finding was negative. There was no

x-ray follow-up of this case, but he withstood 1 year of military duty in apparent good health. The fourth case, a soldier, harbored tubercle bacilli in the sputum and the chest *x*-rays remained negative over a period of 1 year. In addition to his own cases, he cites the work of several French and one Spanish author who reported approximately 20 similar cases. Meersseman appears to have attempted to rule out any local source of tubercle bacilli by stating that at the time of the laryngeal examination he looked for any lesion capable of producing a positive sputum, but he makes no specific mention of having examined the nasopharynx. As an explanation of the above findings, he admits that the pulmonary lesion may exist but be too small to give *x*-ray change or clinical suggestion, but he prefers the theory that it is an elimination of bacilli by a healthy pulmonary parenchyma without concomitant or consecutive tuberculous evolution.

There are numerous reports of the finding of tubercle bacilli in the gastric contents, stools, or sputa of children with no demonstrable pulmonary tuberculosis. Most of these are concerned with tuberculosis of the hilar glands but some refer to cases with no roentgenographic evidence of intrathoracic tuberculosis, some with tuberculous cervical adenitis, others with tuberculosis of bone or joint. In 1927[1a] and again in 1931[1b] Armand-Delille, reporting on the guinea pig inoculation of gastric contents, found that some of the positive cases occurred in children (number not stated) presenting no clinical or roentgenographic evidence of pulmonary disease. Opitz, in 1930[9a] and in 1931,[9b] also reported the results of guinea pig inoculations of gastric contents. Among those in whom tubercle bacilli were discovered, he found 10 patients with negative chest *x*-rays, 8 having tuberculosis of the bone or joints, and 2 having a tuberculous cervical adenitis. Opitz, like Meersseman, mentions many similar cases seen by other physicians. Wallgren[16] reports a like study which yielded a few positive cases apparently lacking roentgenographic evidence of intrathoracic disease. Of the 24 positive culture or guinea pig inoculations of the gastric contents of children reported by Nalbant[8] in 1934, 4 were stated to have no *x*-ray change in the thorax.

It is apparent that the literature is fairly replete with instances in which the presence of tubercle bacilli in the sputum or gastric contents demands an explanation as to the site of their origin. Meersseman[6] apparently searched the lower pharynx and larynx as is common practice. Poulsen[11] refers to the tonsils as a possibility. Kereszturi *et al.*[3] suggest the possibility of a nasopharyngeal source, but no actual demonstration of such an origin is contained in the reports reviewed. We therefore wish to present 2 cases in which such demonstration has been made.

Case Abstracts. CASE 1.—S. K., a white Polish girl, of 18 years, was admitted to the University of Michigan Hospital, June 10, 1936, with the

history of severe colds and sore throats the preceding winter, and having noticed enlargement of the cervical glands 5 weeks before admission. The past history is of importance only in that the tonsils had been removed here 2 years previously and the pathologic sections failed to show evidence of tuberculosis.

Upon examination, the girl was found to be healthy except for the finding of bilateral cervical glands, several being enlarged to the size of a walnut and lying chiefly in the postcervical region. Admission laboratory examination showed only a slight lymphocytosis and a moderately elevated sedimentation rate.

Course in the hospital: The patient was admitted to the general medical service and there a biopsy of one of the cervical glands showed active caseating tuberculosis, after which the patient was transferred to the Tuberculosis Unit for observation and treatment. The routine examination of the concentrated sputum was reported as showing acid-fast organisms typical of tubercle bacilli. This finding was substantiated on four different occasions during the next 4 months. A stereoscopic *x*-ray examination of the chest was negative as was a similar study performed on the former admission 3 years before. While a patient on the Tuberculosis Unit, 4 monthly stereoscopic *x*-ray studies of the chest continued to show no evidence of tuberculosis. In view of the unexplained presence of tubercle bacilli in the sputum, oblique, lateral, apical detail, and Potter-Bucky *x*-ray studies were made and these disclosed no lesion of the lungs, pleura, or mediastinum. Bronchoscopic examination showed no evidence of tuberculous tracheobronchitis as a possible source of positive sputum. Following this period of 4 months' observation, it was decided to transfer this patient to a neighboring sanatorium for further observation. Just before her scheduled departure, in discussing the situation with the patient, we were for the first time impressed with the history of postnasal discharge and the absence of cough. Though we were not aware of a nasopharyngeal lesion as a sole source of tubercle bacilli in the sputum, the localization of the symptoms led us to ask the otolaryngologists to examine this area to see if such existed. They observed a white exudate in the adenoid area, and a smear from this readily showed acid-fast organisms typical of tubercle bacilli by direct examination. The adenoid tissue was removed by the LaForce adenotome and the pathologic report on this tissue was as follows: "Numerous epithelioid tubercles throughout the specimen of adenoid. Some with caseation necrosis." Smears and cultures from that area taken 6 days later showed no tubercle bacilli and nasal washings taken 9 days postoperatively were likewise negative for concentration examination and culture.

This patient has returned for examinations at 6-month intervals since discharge, the last visit being 26 months postoperatively. The postnasal secretion had so diminished after operation and up to the present time that she found difficulty in producing sputum, and that which was produced was negative on three concentrate examinations 2, 18 and 26 months postoperative, and on two cultures, 2 and 26 months postoperative. The severe sore throats she had experienced before operation had entirely disappeared. Roentgenographic examinations of the chest remained negative 26 months postoperative. The cervical glands responded promptly to the first period of bed rest and *x*-ray therapy, and at the last observation could not be palpated.

CASE 2.—A white male, aged 28, was admitted to the University of Michigan Hospital, March 3, 1938. He had been in good health until 9 months before admission when he noticed the sudden onset of pain in the mid-chest, dry cough and temperature elevation to 102°. The chest discomfort, which had been accentuated by respiration, lasted only 1 week,

but the temperature elevation to 101° persisted daily for 4 to 5 months. During this period, he had several *x*-ray examinations of the chest and a bronchogram performed in other hospitals without positive findings. Four months before admission to this hospital, and 4 to 5 months after the onset, he visited a large clinic where a diagnosis of Hodgkin's disease was made on the following findings: 1, *x*-ray at that time showed an enlargement of the upper mediastinum; 2, the cervical, axillary and inguinal nodes had also become enlarged; 3, the appearance of mild abdominal pain and abnormal resistance and tenderness on palpation of the abdomen suggested retroperitoneal adenopathy; 4, the patient reported decrease in the size of the glands and general improvement following the 11 *x*-ray treatments given there; 5, biopsy of a cervical gland was reported as follows: "Lymphnode showing diffuse changes of Hodgkin's disease. Areas of caseation necrosis but no tubercles." This quoted report was made by our pathologist who reviewed the slide after the tuberculous complication had been discovered here.

On admission here, the patient appeared acutely ill with evidence of long-standing illness. The temperature was 100°, pulse 100, respirations 24. There was a biopsy scar in the right supraclavicular fossa. There were multiple pea- to bean-sized anterior and posterior cervical lymph glands, and bilateral olive-sized axillary and inguinal lymph glands which were firm, discrete and non-tender. The chest examination suggested slight increase in the retromanubrial dulness.

The laboratory findings indicated a secondary anemia with suggestive evidence of a lymphoblastoma in the blood smear. Two concentrate examinations and a culture of the sputum were positive for tubercle bacilli as well as three direct smears.

The admission stereoscopic *x*-ray examination of the chest showed a fine stippling over the upper two-thirds of both lungs, which did not have the appearance of tuberculosis and was reported as being strongly suggestive of Hodgkin's disease of the lung parenchyma. There also was enlargement of the hilar shadows. Following our review of the chest *x*-rays, we were unwilling to accept the chest as a source of the tubercle bacilli in the sputum. The only tuberculous lesion it suggested was a localized miliary one, and if so, it was highly atypical in distribution, and, further, it is not our experience to expect bacilliferous sputum in such cases.

With the experience of the first case in mind, reinvestigation of this patient readily revealed the history of a profuse postnasal discharge which had been present for about 3 months. The laryngologist reported a small ulcer of the larynx and more importantly, an ulceration in the adenoid area about 17 mm. in diameter. A biopsy of the tissue in that area is reported as follows: "Numerous discrete and confluent epithelioid tubercles with caseation necrosis scattered throughout the tissue submitted. Small localized ulceration of the nasopharyngeal mucosa. Lymphoid hyperplasia. No evidence of Hodgkin's disease." Stain of the tissue readily showed acid-fast bacilli. The patient's course was rapidly downhill with daily temperature elevations to 102° to 103°. A week before death he developed intestinal obstruction, but in view of the prognosis and the patient's poor general condition, an operation was not performed. He died 22 days after admission. An autopsy was refused.

Comment. Two cases are cited to show that tuberculosis of the nasopharynx is a source of tubercle bacilli in the expectoration. In the first case, this was the only source discovered, and surgical removal of the focus relieved the patient of the symptoms and the infectious sputum, and more importantly, removed the necessity

for further treatment or isolation. The second case is cited mainly to support the fact that this area is a source of bacilliferous sputum and to call attention to the fact that whenever tubercle bacilli in the sputum tend to refute the clinical and roentgenologic diagnosis of a non-tuberculous condition, the nasopharynx should be investigated.

Both patients reported that the sputum was not brought up from the lungs by cough. In the first patient, the posterior cervical glands were shown by biopsy to be tuberculous. Since this chain drains the posterior nasopharyngeal area, it is believed that the adenoid lesion was a source of the tuberculous adenitis. The presence of these conditions, *i. e.*, postnasal discharge and posterior cervical adenitis should further direct attention toward the posterior nasopharynx in patients with a positive sputum from an unidentified source.

When the case records of the same material reported by Weller,[17] later by Magee,[5] and more recently by Pollard and Combs[10] were reviewed, it was found that enlargement of the posterior cervical lymph glands was not an invariable concomitant of tuberculosis of the adenoids.

It has been pointed out that the combination of a positive sputum and negative chest *x*-ray is not a rarity in the literature. It is not maintained that nasopharyngeal infection may account for all or even a majority of these instances, but it is shown that it may account for some. The incidence of tuberculosis in the adenoid tissue has not been studied as thoroughly as that of the tonsil. In 1917, Crowe *et al.*[2] showed by microscopic examination that 1.7% of the adenoids removed during 1000 routine tonsillectomies and adenoidectomies were tuberculous. Scarff and Whitby,[14] in 1929, inoculated guinea pigs with macerated adenoid tissue from 50 patients having routine adenoidectomies and, in 2 of these, positive results were found. It is interesting that these were the only 2 patients in the group with a tuberculous cervical adenitis.

It is not believed that the failure to consider this lesion will be a common source of error in clinical work but since the suspected area is accessible to the examiner by inversion of the laryngeal mirror, it is suggested that it be made routine in all cases of tuberculosis, and that it certainly never should be omitted in patients with postnasal drip and cervical adenitis. The examination should precede the bronchoscopic search for source of bacilli when the lung appears to be free of tuberculosis or to be adequately controlled by collapse therapy.

Conclusion. Two cases are presented to demonstrate that tubercle bacilli in the sputum may arise from the posterior nasopharynx; in 1 of these, this appeared to be the sole source of bacilli.

When the chest roentgenogram shows no evidence of tuberculosis, or when the changes present do not serve as a satisfactory explana-

tion as to the source of tubercle bacilli in the sputum or gastric contents, a tuberculous lesion in the posterior nasopharynx as well as one in the tracheobronchial tree should be suspected as the site of origin of the bacilli.

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HEMORRHAGIC DIATHESIS WITH PROLONGED COAGULATION TIME ASSOCIATED WITH A CIRCULATING ANTICOAGULANT.*

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The possibility that hemorrhagic diatheses which are associated with a prolonged venous blood coagulation time may be due to the existence of a circulating anticoagulant has been suggested on several occasions.[13,29] However, as far as can be ascertained from the literature the presence of such an anticoagulant has never been actually observed in the plasma of a patient. Indeed in true hemophilia this hypothesis has been investigated and found to be untenable.[26] The purpose of this communication is to report the history, necropsy findings and certain investigations on a patient with hemorrhagic diathesis in whom a prolonged coagulation time appeared to be associated with the presence of a circulating anticoagulant.

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Case Report. *A 61-year-old mulatto male with 8 months cachexia, recent hemorrhagic manifestations, generalized lymphadenopathy and prolonged coagulation time, died of postoperative hemorrhage following excision of lymph node.*

Present Illness. J. S. (No. 922309, B. C. H.), a 61-year-old colored married American dining car waiter was admitted on December 6, 1938, complaining of pain in the left flank and left upper quadrant of 3 days' duration. This was cramplike but dull in nature and associated with febrile and chilly sensations. On the day before admission he vomited twice and had a severe epistaxis. For 8 months he had not been feeling well, suffering from dyspnea on exertion, mild orthopnea and ankle edema. During the 7 months prior to admission, after being unable to work for 6 months, he was digitalized and worked for the 1 month without distress.

Family History. Father died of "cancer of rectum." One sibling is thought to have died of tuberculosis many years ago. There was no history of hemorrhagic disorders.

Past History. Until the present illness the patient was remarkably well, working faithfully as a dining car waiter. During the last 7 years he suffered very slight epistaxes about once a year and had a hacking nocturnal cough. Thirty years ago he had gonorrhea and 6 years ago following a routine blood test he was given a few intramuscular injections the nature of which was not discovered but which produced a transitory attack of jaundice. There was no history of alcoholism or use of tobacco. In his occupation he was exposed to brass and silver polish and the usual fumes and smoke of the railroads. During the last year his weight fell from 75 to 64 kg. He was married twice. One daughter by his first wife is living and well.

Physical Examination. On admission he was acutely ill, fairly well developed, somewhat undernourished and moderately dyspneic. Temperature 39° C., pulse 100, respirations 24. Blood pressure 135/70. There were diminished resonance and moist râles at both lung bases. The heart was slightly enlarged to the left by percussion, with a gallop rhythm and precordial systolic murmur. The abdomen was tender in the left flank as was the left costo-vertebral angle. A small firm lymph node was palpable in the right supraclavicular space and both inguinal chains were moderately enlarged, the nodes being firm but freely movable.

Laboratory Findings. (A large amount of laboratory work was done on this patient and all duplicate findings are omitted.)

Urine. Microscopic hematuria and slight albuminuria were present constantly. The highest specific gravity of the urine was 1.026. On admission ureteral catherization revealed that red cells were coming from left kidney.

Blood (Hematology). On admission the patient's hemoglobin was 75% (Sahli) (11.39 gm.), the red blood cell count 4.1 million, the white blood cell count 11,200; with 60% neutrophils, 24% lymphocytes and 16% monocytes. The patient's hemoglobin steadily declined, the only known blood loss being from hematuria and hematomata; on the 18th hospital day the hemoglobin was 36% (5.62 gm.). At this time the white count was 7900 with 65.5% adult neutrophils, 7.5% "bands," 3.5% eosinophils, 8.5% lymphocytes, 15% monocytes. There were no significant changes in these findings except for those transitory increases produced by transfusion and iron therapy. The mean corpuscular volume of the red cells averaged 90.0; the reticulocyte percentage 1.5. The corrected sedimentation rate (Rourke-Ernstene method) for whole oxalated blood was 1.2 mm./min. and for defibrinated blood 0.45 mm./min. Special hematologic findings are summarized in Table 1.

Blood (Chemistry). The non-protein nitrogen varied from 22 to 40 mg./100 ml. plasma. The total protein was 6.79 gm./100 ml. plasma and a partition of the protein showed the albumin to be 3.06 gm., the globulin 3.22 gm.

and the fibrin 505 mg./100 ml. of plasma. The fasting blood sugar and glucose tolerance curves were normal. The blood calcium was 8.74 mg./100 ml. serum, and inorganic phosphate 3.09 mg./100 ml. serum. The lipid constituents were essentially normal; total lipids 531 mg.; phospholipid 9.3 mg.; cholesterol 210 mg., free 81 mg. and ester 128 mg.; all per 100 ml. plasma. The blood cevitamic acid level was 0.55 mg./100 ml. plasma which while low is above the values found in scurvy. Takata Ara reaction ++++.

TABLE 1.—RÉSUMÉ OF LABORATORY FINDINGS PERTAINING TO HEMORRHAGIC DIATHESES.

Platelet count (direct)	335,000 to 937,000/c.mm.
Platelet morphology	Normal
Bleeding time (Duke)	½ to 3½ minutes
Coagulation time (Patek and Stetson)	68 to 90 minutes
Clot retraction	Normal
Tourniquet test	Normal
"Prothrombin" time (Quick)	Normal
Serum calcium	8.74 mg./100 ml. serum
Plasma fibrinogen	505 mg./100 ml. plasma
Plasma vitamin C	0.55 mg./100 ml. plasma

Miscellaneous. The Hinton test on the blood serum was positive. No acid-fast bacilli were demonstrable in the sputum. The stools were positive on rare occasions for occult blood. Phenolsulphonephthalein test showed 60% excretion of the dye in 2 hours following its intravenous injection. Blood group O (Moss IV). Hippuric acid excretion following sodium benzoate ingestion was 50% of normal on one occasion, normal at a later period.

Roentgen Ray Findings. Congestive changes were apparent in both lung bases and there was marked enlargement of the hilar glands. There was a hypertensive deformity of heart and the aortic knob was sclerotic. Both intravenous and retrograde pyelography were negative. Dilatable constrictions of third portion of duodenum and several other areas along small intestine and large intestine suggested pathologic changes extrinsic to gastrointestinal tract. The spine, humeri, skull and femora showed no abnormalities. There were calcified lymph nodes in the right groin.

Clinical Course. The admission symptoms subsided in 24 hours and at no time thereafter was there any recurrence of fever nor did the patient complain again of pain in left flank. Repeated physical examinations showed no noteworthy changes. He did fairly well on maintenance doses of digitalis until the 14th hospital day when he bumped his left hip against a table and developed a hematoma the size of a grapefruit. On his 23d hospital day he was transfused with 500 ml. citrated blood from a compatible donor without untoward reaction and was placed on ferrous sulphate 0.8 gm. daily. On his 33d hospital day he began to bleed moderately profusely from his right nostril. This hemorrhage was controlled by a pack impregnated with "steer globulin substance"[30] changed daily for 3 days when bleeding ceased. From this time on however the patient's condition gradually grew worse and he suffered two spontaneous hematomata in the left calf and right hip respectively. On the 64th hospital day because of the obscurity of diagnosis it was decided to biopsy the sternal bone marrow and to excise the right supraclavicular lymph node. There was no excessive bleeding at the time of operation, but postoperatively he bled from the supraclavicular incision into his neck and superior mediastinum, packing of the wound and a 500 ml. transfusion proving of no avail in stopping the hemorrhage and he died on his 66th hospital day.

Biopsy of Sternal Bone Marrow and Supraclavicular Lymph Node. The sternal bone marrow was essentially negative on gross and microscopic

FIG. 1.—Cut section of lymph nodes at bifurcation of trachea.

FIG. 2.—Cut section of lymph nodes surrounding duodenum.

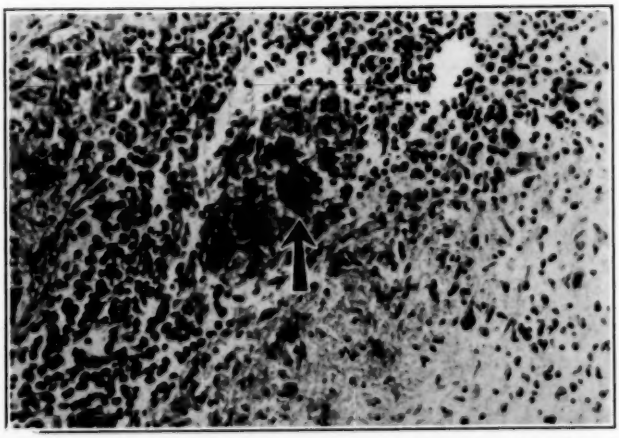

FIG. 3.—Small active tubercle from pancreatico-duodenal lymph node. (× 186.) Note histiocytic center with Langhans giant cell (arrow) and surrounding lymphocytic reaction.

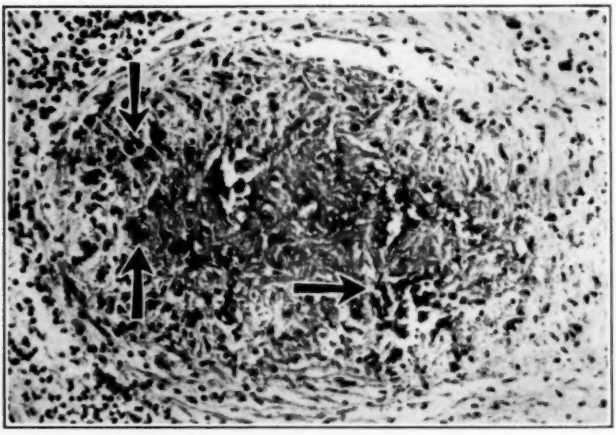

FIG. 4.—Small healed tubercle from pancreatico-duodenal lymph node. (× 186.) Note hyalinized center of nodule containing pigment (arrows) and surrounded by fibrous tissue.

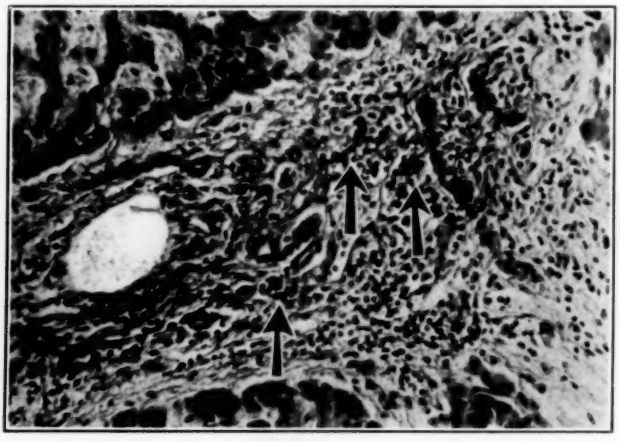

FIG. 5.—Portal space of liver. (× 310.) Note deposits of pigment (arrows) in connective tissue.

examination. The supraclavicular lymph node was 1 by 1 by 2 cm. in size, firm in consistence and mottled dark gray and black in color on cut section. On microscopic examination, it showed marked anthracosis and fibrosis, and lesions with hyalinized centers surrounded by lymphocytic reaction suggesting healing tubercles. An acid-fast stain for tubercle bacilli revealed no such organisms. However, when a guinea pig was inoculated with some of the macerated tissue, it developed manifest tuberculosis.

Necropsy (performed 15 hours after death). The body length was 173 cm. and the weight 50.1 kg. There were fresh ecchymoses over the swollen neck extending on to the upper anterior chest. In the right supraclavicular fossa, there was a wound, 3.5 cm. in length, packed with blood-soaked gauze, and another over the midsternum, 4.5 cm. in length, which was sutured and apparently healing. There was no peripheral edema.

On opening the thorax, the anterior *mediastinum* showed dark purple hemorrhagic areas extending from the sternal notch to the superior border of the pericardium. Firm fibrous adhesions were found in both pleural spaces. In the pericardial cavity a few thin fibrous adhesions and 3 to 4 ml. of clotted blood were present. A small subepicardial hemorrhage was observed posteriorly. The *heart* and *lungs* appeared otherwise negative. The heart's blood was sterile on culture. In the pelvic region, the parietal and a portion of the visceral *peritoneum* was conspicuous by its dark gray almost black color. This was attributed to a previous retroperitoneal hemorrhage, the source of which, however, could not be located.

The *liver* was slightly increased in weight (1600 gm.) but otherwise presented no gross abnormalities. A single brown granular stone 2 cm. in diameter was found in the *gall bladder*. The *aorta* showed a mild degree of atheromatous change. The femoral *bone marrow* was a mottled mixture of dark red brown tissue and fat, the vertebral marrow was pale red brown and the tibial marrow was entirely fatty.

The most striking pathologic change to be found at autopsy was in the lymphatic system. Every lymph node that was available at postmortem examination was involved by a granulomatous lesion to a greater or less degree. The inguinal nodes were apparently most affected being grossly caseous and containing several areas of calcification. The tracheo-bronchial and hilar nodes in the chest and the pancreatico-duodenal, periportal and mesenteric nodes in the abdomen were all discretely enlarged varying from 0.5 to 5 cm. in diameter. The nodes in the chest were of rubbery consistence and cut with a cartilaginous gritty sensation. Those in the abdomen were slightly softer. All showed on cut section a speckled dark green and gray surface with the configurations of green granite. (See Figs. 1 and 2.)

The remainder of the organs were grossly negative.

Microscopic. The chief lesions were in the lymphatic system. Every node examined presented the histologic evidences of tuberculosis. The inguinal nodes were apparently most affected, being composed largely of caseous and calcareous deposits. The supraclavicular, tracheo-bronchial, hilar, pancreatico-duodenal, periportal and mesenteric nodes all contained focal areas of fibrosis and caseation necrosis (Fig. 4) with deposition of calcium and cholesterin. In selected regions of these nodes typical active tubercles could be demonstrated. These showed a histiocytic center with Langhans giant cells and surrounding lymphocytic reaction (Fig. 3). Stains for acid-fast bacilli however revealed no such organisms. In the remainder of the organs there were no lesions absolutely characteristic of tuberculosis. In the lungs there was a focal area of fibrosis which was consistent with a healed tubercle.

The amount and distribution of anthracosis in the tissues was greater than that usually seen. Carbon pigment was present in all the lymph nodes except those in the inguinal region. The lungs contained unusually large

amounts. Pigment with the appearance of carbon was also present in the connective tissue of the portal spaces of the liver (Fig. 5) and in the reticuloendothelial cells of the spleen and vertebral bone marrow. There was slight periportal cirrhosis in the liver.

The tibial and vertebral bone marrow presented a moderate hyperplasia of both the erythropoietic and leukopoietic elements.

Both kidneys contained focal areas in which most of the tubules were replaced by fibrous connective tissue in which there were infiltrating lymphocytes. The tubules remaining in these areas contained darkly basophilic hyaline material in the lumens.

The sections were examined for silica with the polarizing microscope. In the lung nodule previously described and in the hilar and cervical lymph nodes, rare doubly refractile crystals consistent with the appearance of silica were found. The amount however was within normal limits and far below that seen in silicosis.

Final Postmortem Diagnoses. Generalized tuberculosis of lymph nodes. Anthracosis of lymph nodes, lungs, liver, spleen and bone marrow. Findings consistent with a hemorrhagic diathesis (hemorrhage from operative wound, old retroperitoneal hemorrhage, subepicardial hematoma and hemopericardium). Hyperplasia of bone marrow. Fibrous pleuritis, bilateral. Healed pyelonephritis, bilateral. Chronic cholecystitis and cholelithiasis. Slight biliary cirrhosis of liver.

Generalized lymph node tuberculosis is a rare condition. In this patient, as in most of the cases reported, the portal of entry of the organism is unknown. The subject has recently been reviewed by Miller.[20]

Investigation of the Coagulation Defect of Patient's Blood. The results of the routine laboratory tests are condensed in Table 1. The most striking abnormality was the prolonged blood coagulation time, ranging from 68 to 90 minutes on numerous occasions. This finding was clearly not associated with fibrinogen, prothrombin or calcium deficiency and the platelets were increased rather than diminished. The laboratory examinations resembled closely those presented by true hereditary hemophilia. There was, however, nothing in the patient's past or family history to suggest such a diagnosis.

Certain standard procedures developed during the past 3 years in this laboratory[26,27] for the study of the coagulation defect in hemophilia were applied to this patient. A transfusion of 500 ml. citrated blood from a normal compatible donor was given the patient but was followed by no change in the coagulation time of his blood. This is strikingly different from the fall in the coagulation time of patients with hemophilia produced by such transfusions of whole blood or plasma.[23,26] Using the standard technique previously described,[26,27] it was observed that the addition to 2 ml. of the patient's blood of various amounts of normal plasma* and of

* Hereafter the word plasma refers to the preparation obtained by adding sodium citrate to whole blood so that the final citrate concentration is 0.25%. centrifuging at 2000 r.p.m. for 30 minutes, filtration through No. 2 Whatman paper and then through a Berkefeld V filter.

certain derivatives of plasma known to reduce the coagulation time of hemophilic blood did *not* reduce the coagulation time of this patient's blood. It was found, however, that the addition of 0.4 ml. of an 0.5% solution of calcium chloride did reduce the clotting time of 2 ml. of the patient's blood from 68 to 27 minutes *in vitro*. Larger amounts of calcium chloride had no further effect.

When small amounts of the patient's plasma were added to 2 ml. of normal human blood the coagulation time of the normal blood was increased from 7 to 28 minutes in one instance and from 11 to 32 minutes in a second (Table 2). This anticoagulant effect of the

TABLE 2.—EFFECT OF CALCIUM ION, PATIENT'S AND HEMOPHILIC PLASMA ON THE COAGULATION TIME OF NORMAL BLOOD.

Ml. of materials added to 2 ml. of normal blood.			
Patient's plasma.	Hemophilic plasma.	0.5% calcium chloride.	Coagulation time of mixture in minutes.
0	0	0 (control)	7
0.01	0	0	10
0.05	0	0	19
0.10	0	0	28
0.20	0	0	42
0.10	0	0.10	16
0.20	0	0.10	16
0	0.10	0	9
0	0.20	0	15
0	0.10	0.10	6
0	0.20	0.10	6
0	0	0.10	5

patient's plasma on normal blood was striking in dilutions of 1 to 20 and 1 to 40 and still quite apparent at a dilution of as great as 1 to 200. The addition of calcium chloride partially inhibited this anticoagulant effect as it partially reduced the clotting time of the patient's own blood. When these observations were repeated with hemophilic plasma on normal blood no anticoagulant effect of the hemophilic plasma could be demonstrated except in dilutions of 1 to 10 when the characteristic prolongation due to citrate ion intervened.

A preliminary* investigation into the nature of the anticoagulant in this patient's plasma was attempted. The slightly increased plasma globulin and fibrinogen levels of this patient suggested the possibility of liver damage. Because of this the question arose as to whether this patient might have an excessive amount of heparin in the circulating blood. Chargaff[5] has shown that heparin when present in blood may be neutralized by the addition of salmine sulphate. When this patient's blood was titrated with this pro-

* 200 ml. of this patient's blood plasma were obtained during life and were "lyophilized" and some of these observations were made postmortem on such plasma after rediluting to the original volume with distilled water.

tamine* in amounts varying from 0.001 to 0.1 mg. per 2 ml. of blood there was *no* effect on the coagulation time except that with increasing concentration of salmine sulphate the intrinsic anticoagulant effect of this substance became manifest. On the basis of Chargaff's report the amount of salmine sulphate used in the above experiment should have served to neutralize concentrations of heparin varying from 100 to 10,000 inhibitor units per kg. body weight. We accepted these observations as evidence that the anticoagulant substance was not heparin.

The anticoagulant was relatively thermostabile, resisting a temperature of 37.5° C. for 72 hours and 61° C. for 10 minutes. Ultrafiltrates of the patient's plasma through cellophane membranes under a positive pressure of 20 cm. of mercury for 24 hours contained no anticoagulant activity. This would indicate that the anticoagulant is not a simple diffusible substance.

Dialysis of the plasma against distilled water for 8 days at a temperature of 8° C. yielded an euglobulin fraction and a water-soluble protein fraction neither of which had anticoagulant action. Unlike preparations from normal plasma,[18,27] the euglobulin possessed no clot promoting activity for hemophilic blood. When the globulin fraction was prepared by diluting the plasma with 10 times its volume of distilled water and precipitation with 1% acetic acid at pH 5.6, it was found to be neither coagulant nor anticoagulant for hemophilic blood. Such preparations from normal plasma have marked clot accelerating power for hemophilic blood.[27] Removal of the proteins from the plasma by ethyl alcohol[15] left an alcohol residue which when evaporated to dryness *in vacuo* and redissolved in water showed *no* anticoagulant activity.

Continuous ethereal extraction for 24 hours employing a Soxhlet extractor did not yield an active extract but the residue retained its full anticoagulant activity.

No fibrinolysin was demonstrable in the patient's plasma or serum.

Discussion. The patient described represents an individual with hemorrhagic diathesis associated with a prolonged coagulation time. This finding is relatively uncommon and the conditions in which it occurs have recently been reviewed by Pickering.[28] In only true hemophilia is such decreased coagulability of the blood an invariable finding. The clinical and genetic characteristics of this disease are well described[1,2] but the pathologic physiology still remains incompletely understood.[16c,27] The individual reported here can be sharply differentiated from hemophilia on the basis of the obviously dissimilar clinical picture and certain established physiologic criteria. The latter include the failure of blood transfusion to reduce the coagulation time of the patient's blood *in vivo* and the failure of normal human plasma and certain of its active components to

* Kindly furnished by Eli Lilly and Company, Indianapolis, Indiana.

reduce the coagulation time of the patient's blood *in vitro*. These same criteria also differentiate the patient reported here from the female patient of Joules and Macfarlane,[17] who presented a prolonged blood coagulation time which was reduced *in vitro* by the addition of normal human plasma. In certain other hemorrhagic diatheses, increased coagulation time has been reported occasionally. Among these are biliary obstruction, biliary fistula or liver disease.[4] The decreased coagulability of the blood in these conditions appears to be associated with a vitamin K deficiency and a consequent reduction of prothrombin in the blood.[3,31] Lack of fibrinogen may be responsible for prolongation of the clotting time. This is usually congenital[19] but may be associated with hepatic insufficiency.[24] In thrombocytopenia prolonged clotting time has been reported in surprisingly rare instances[34] in spite of the decreased coagulability said to accompany artificial removal of the platelets.[7,8] In 1915, Hess[14] reported a patient with hemophilia which he suggested was due to calcium deficiency on the bases of a negative calcium balance and of the shortening of the coagulation time of the patient's blood produced by the addition of calcium chloride *in vitro*. This condition he termed "hemophilia calcipriva." However, the patient was only 6 years old, at which age a negative calcium balance is not unusual and the direct effect of the addition of calcium has been repeatedly observed in true hemophilia.[16a,18b] In addition, it has been observed in the coagulation of dog's blood that levels of calcium sufficiently low to interfere with clotting were far below those compatible with life of the animal.[32] It is doubtful therefore that "hemophilia calcipriva" can exist. The patient reported here may be differentiated from all these conditions by the laboratory observations. The "prothrombin" time, icteric index, blood fibrinogen, serum calcium were all within normal limits. The blood platelet counts were somewhat elevated. In spite of a slightly elevated plasma globulin level, the liver function as tested by sodium benzoate detoxification was grossly normal.

At times there has been reported in connection with certain conditions a hemorrhagic diathesis with a prolonged coagulation time unassociated with prothrombin, fibrinogen or calcium deficiency. These include tertiary syphilis,[25] multiple myeloma[10] and uremia.[9] The patient reported here had a positive blood test for syphilis but at autopsy there was no anatomic evidence of this disease. Multiple myeloma was not present at autopsy and during life it was impossible to demonstrate Bence-Jones protein in the urine. Repeated blood non-protein nitrogen determinations were within normal limits.

In the consideration of the nomenclature of the condition which this patient presented, the term "pseudohemophilia" suggests itself. This is a term which has been applied to hemorrhagic diatheses associated with a prolonged coagulation time but equally

to those resulting from fibrinogen deficiency[28] and to those resembling hemophilia.[19] In addition, it has been very frequently applied to Glanzmann's hereditary hemorrhagic thrombasthenia,[11,12,21,33] a disorder characterized by normal or prolonged bleeding time and by normal clotting time. This term therefore is ambiguous and misleading.

In all probability the most significant laboratory finding to be related to the prolonged coagulation time of the patient reported here is the demonstration of the presence in his circulating blood of a substance interfering with the completion of the normal clotting mechanism.* Whether this anticoagulant is identical with the "inhibiting substance" of Howell[16a,b] cannot be said. It does, however, possess the thermostability and non-diffusibility of that "substance."[16a] As far as the demonstration of the existence of some "inhibiting substance" is concerned, the data given in the text exhibit this situation even more comprehensively than the usual *in vitro* exposition[16a,19] of antithrombic activity since the inhibition of blood coagulation occurs *even when calcium, prothrombin and fibrinogen are present in adequate amounts in the system.*

The unusually generalized anthracosis extending even into the liver observed at postmortem examination appears to be the only anatomic finding which might bear a relationship to the pathologic physiology of this patient. What this relationship is can only be speculation at the present moment. As far as the generalized lymph node tuberculosis is concerned, a review of the literature has revealed that several cases presenting purpura hemorrhagica have been reported[6] but none in which the coagulation time is mentioned as being prolonged. Minot[21] has reported hemorrhagic diathesis with prolonged coagulation time in a patient with miliary tuberculosis.

To recapitulate then, the patient reported here was a 61-year-old mulatto dining-car waiter presenting an 8-month history of progressive cachexia. Physical examination revealed slight lymphadenopathy and the ordinary degenerative findings of his age group. The routine laboratory studies revealed serologic syphilis, microscopic hematuria, moderate hypochromic anemia, occasional melena and hematemesis and prolonged coagulation time. There was roentgenographic evidence of hilar enlargement in the chest and multiple lesions extrinsic to the gut in the peritoneal cavity. During his hospital course he developed 2 traumatic hematomata, 2 spontaneous hematomata, and a severe epistaxis. He died of postoperative hemorrhage following excision of a cervical lymph node and

* Dr. W. H. Howell[16d] after kindly reviewing the data presented by this patient has pointed out that in addition to the presence of an anticoagulant a deficiency of "globulin substance"[24] or "plasma thromboplastin"[16c] may have contributed to the prolonged coagulation time.

biopsy of sternal bone marrow. The essential postmortem finding was generalized lymph node tuberculosis and anthracosis. The most interesting observation concerning the patient was the anticoagulant activity of his plasma for normal blood. The anticoagulant is a thermostabile, non-diffusible and non-ultrafilterable substance.

Summary and Conclusions. A patient with hemorrhagic diathesis is reported in whom a prolonged blood coagulation time was associated with the proven existence of an anticoagulant in his blood plasma. The postmortem examination revealed generalized lymph node tuberculosis and anthracosis. The relationship of these findings to the pathologic physiology involved is not established.

It is quite possible that the presence of a circulating anticoagulant may be associated with the prolongation of the blood coagulation time found in other conditions. It would seem advisable therefore that in such instances the anticoagulant properties of the patient's plasma should be investigated.

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TRICHINOSIS.

A Study of 23 Cases.

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The subject of trichinosis has recently attracted considerable attention. This interest has come for the most part from an effort of the United States Public Health Service to obtain data regarding the distribution of the parasite in the general population and from their desire to offer a program for the effective control of the disease. A general impression of the incidence of trichinosis may be obtained from the Annual Report of the Surgeon-General of the Public Health Service of the United States.[2] For example, an examination of 2000 diaphragms in and around Washington, D. C., showed that 17.1% were positive for trichinæ. In a series comprising material received from hospitals selected at random in various parts of the United States, 375 diaphragms were examined and 18.8% were found to be positive for trichinæ. It is interesting to note that of 98 diaphragms in a series of individuals who met death from accidents, 15.3% were positive for trichinæ. In order to determine the distribution of trichinosis throughout the country as a whole, sections of diaphragms were obtained from states in which the disease had never been reported. From Arizona 24 diaphragms showed an incidence of 12.5%; of 77 diaphragms from New Hampshire, 16.9% were affected; and an examination of 26 diaphragms from Oklahoma disclosed the trichinella in 26.9%. At the Milwaukee County Hospital 10 diaphragms were examined and the incidence was found to be 20%.

These studies leave no doubt that trichinosis is much more prevalent than it is usually thought to be. A general review of the work done on the subject shows that at postmortem examination about 1 individual in 6 has positive evidence of the disease. It may be concluded, then, that most patients who have had trichinosis either gave no clinical evidence of the disease or the signs and symptoms were misinterpreted and an erroneous diagnosis made. With a view of obtaining further data upon the question of trich-

inosis in this vicinity we have collected all the cases diagnosed clinically at this hospital for the past 8 years.

Reports such as those given by Aldridge[1] and Carlson[7] show that outbreaks of trichinosis occur in certain districts. In these epidemics the cases appear to show the classic characteristics more definitely than the sporadically collected cases. The series of cases reported here were diagnosed from 138,060 patients entering the hospital from January 1, 1931, to January 1, 1939. In many instances the diagnosis had been made before entrance, but in others the condition had been mistaken for some other disease.

The main clinical data of the series are included in Table 1. In the 23 cases presented here, the age incidence ranged from 17 to 57 years. Only 2 patients were 50 years old or over. This is in distinct contrast to Pote's[27] series. He reported that of 163 cases, 32.2% were in the age group of 15 to 50 years, while 61.9% were over 50 years. In our series, 12 of the patients were males and 11 were females; sex differences seemed to have no significance. The patients came from all walks of life; 2 were physicians, 1 a nurse, and most were laborers. Occupation had no importance, for a disregard of the danger of eating poorly prepared food does not characterize any particular class. In 14 cases there was a history of having eaten inadequately cooked pork, raw hamburger sandwiches or hamburgers obtained from stands along the road-side. A cursory glance at our table shows that most of the patients developed trichinosis during the warmer periods of the year when road-side stands are operated for the convenience of the motoring public.

Marked leukocytosis occurred in all but 2 cases (Cases 3 and 18). Both of these patients recovered. Eosinophilia was definitely established in all but 1 (Case 2), and this patient died. The maximum eosinophilia was 73% (Case 16) and the minimum 4% (Case 2). An autopsy done in the latter case established the diagnosis of trichinosis.

Trichinosis does not always present a uniform clinical picture. The forms showing the classic picture are recognized easily and promptly while in the unusual types, such as those in which essential internal organs are involved, diagnosis may be very difficult. Most of the cases reported here were of the common type and began with nausea, vomiting, abdominal distress and diarrhea which came on within several hours after having eaten the questionable meat. Occasionally these gastro-intestinal disturbances were lacking, and frequently they were attributed to some disorder entirely unconnected with trichinosis. Pain in the muscles and joints and swollen eyelids were by far the most outstanding clinical features noted. Fever was not marked in any case, and frequently it was completely absent. Of the 23 cases reviewed here, 7 had few symptoms, 6 had symptoms of a moderate degree of severity and in 10

the signs and symptoms were well developed and the patients were very sick. The conditions most commonly confused with trichinosis in this group of cases were sinus infection, influenza, rheumatic fever, la grippe and acute nephritis. In 3 cases a more serious diagnosis such as typhoid fever or tularemia was suspected. A diagnosis of acute nephritis was made before the true condition was recognized in 2 patients. The disease was identified in most cases when the high eosinophil count was found in association with the other features of trichinosis. When any doubt existed a muscle biopsy was done and the Bachman test was resorted to in a number of cases.

Most of the patients were free of symptoms after 2 or 3 weeks. However, the course was more prolonged in several of the more severe cases. In 1 case, thrombophlebitis of the left femoral vein developed and the patient was confined to the hospital for several months. Of the 23 cases, 2 died and 21 recovered completely, a mortality rate of 8.6%.

It did not appear to us that the treatment after entrance to the hospital had much influence upon the course of the disease. When the larvæ become encysted in the muscles or organs of the body very little can be accomplished in a specific way in treatment. We were impressed with the fact, however, that the individuals seen in the earlier stages by a physician who was thoughtful enough to clean out the gastro-intestinal tract had a milder course and recovered more promptly. Anthelmintic measures such as male-fern and santonin were employed in some of our cases without much effect. Copious amounts of fluid and a highly nutritious diet were given. Palliative measures such as sedatives and acetyl salicylic acid were used for the relief of pain and headache. In some cases intravenous injections of calcium gluconate, mercurochrome and gentian violet were employed, but the value of these therapeutic procedures seemed very doubtful to us. Fantus[12] recommends a preliminary purge with mercurous chloride (0.2 gm.) followed in 8 hours by a dose of magnesium sulphate. He repeats this treatment several days in succession in order to sweep out the worms that have not invaded the submucosa. Good results were reported by Grove[14] who used antimony and potassium tartrate in a 2% solution intravenously. Our clinical experience from the therapeutic standpoint coincides with the experimental work done by Miller, McCoy and Bradford[25] who tested the efficacy of many of these intravenous treatments experimentally. They concluded that no demonstrable therapeutic effect was obtained. On the basis of their work they questioned the rationale of intravenous injections of such substances as gentian violet, antimony, potassium tartrate, neoarsphenamine, metaphen and iodine.

Discussion. It is generally agreed that the high incidence of trichinosis observed at autopsy is not reflected clinically. As Pote[27]

aptly points out, a study of each of his 163 positive cases revealed that infestation did not seriously affect the health of the individual or the course of the terminal sickness. However, from our own series of cases and from a study of the literature there can be no doubt that in some instances trichinosis is a grave clinical condition that may cause weeks of disability or even death.

From the data published by Hall and Collins[17] it is apparent that measures used to control trichinosis during the past 50 years have failed. There is no microscopic inspection of pork in this country, and fresh pork or pork products to be eaten after cooking by the consumer are not processed. As pointed out by Hall the government inspects only about 70% of the meat sold. In packing houses operated under Federal supervision all pork products that are to be used without cooking are processed to kill the trichina, but about 50% of the pork goes to the consumer in raw state. Lack of proper processing is considered to be a fertile source of trichinosis, and another common cause is pork products prepared without supervision in small local slaughter houses or on farms. Since the United States government long ago gave up attempts to eliminate trichinosis by microscopic examination of pork, considerable meat has been marketed in this country without any inspection whatever. Fresh pork sausage, smoked hams and shoulders, bacon and such products as smoked sausage, boneless loins, and coppa should be cooked until they are well done throughout before consumption. Thirty minutes to the pound is an approximate guide to sufficient cooking for large, thick cuts of pork. Pork products of the sort customarily eaten without cooking by the consumer, largely various kinds of dry or summer sausage are entirely safe to eat without cooking if prepared under Federal or other competent inspection. The pork used in such products is especially processed by cooking, freezing or curing to destroy any trichinæ that may be present.

Until recent years no steps were taken to solve the problem of trichinosis, but the latest work of Hall and his associates seems to have laid a foundation for subsequent control. A summary of the problem may be taken from Hall's last report.[16c] Human trichinosis rests, he states, upon the basis of swine trichinosis and swine trichinosis rests primarily upon uncooked pork scraps in garbage, table scraps and swill, the rat having only a minor and not definitely ascertained rôle in swine trichinosis. He insists that the basic solution of the problem lies in keeping raw and inadequately cooked pork scraps out of the feed of swine. His suggestion for the control of trichinosis calls for improvement in methods of raising swine, these improvements to be specified by the packers as requirements to be met by swine growers before their products can be marketed. The elimination of the dangerous practice of feeding raw or inadequately cooked pork scraps to swine would play an important rôle in the control of trichinosis. He warns that useless publicity or ill-

advised legislation are not the correct control measures for trichinosis and that the packer and swine grower should be called upon to coöperate in the solution of the problem.

Etiology. The trichinella spiralis, the parasite which causes the disease, exists in an encysted form in the muscles of the rat, pig, wild boar, dog, fox, bear and badger. Lower animals are the normal hosts for the organisms; human infection is accidental. When a human being ingests trichinous meat, the cyst wall is digested in the stomach and the worms pass actively into the small intestine where they reach maturity in about 3 days. The females are fertilized, and on about the seventh day they burrow into the mucosa of the intestine, allowing embryos to escape into the tissues and lymph spaces. It is not definitely known how the embryos get from the intestinal wall to the muscles, but it is probable that they pass through the lung into the general circulation because of the fact that they are smaller than a red blood cell. By the tenth day the embryos are usually found in the muscles where they grow to maturity, often becoming as large as 1 mm. in length. They coil up and become completely encysted by the twelfth week and may live in this state for 20 or 30 years. When the meat containing these cysts is eaten the cycle is started again. There is little chance for further development once the embryos become encysted in the muscles of a human being. It is only among lower animals that the worms develop from generation to generation.

Infection with the trichinella spiralis is common in hogs throughout the world. Ransom[28] tabulated the results of the United States Government trichina inspection of over 8,000,000 hogs exported during 9 years between 1898 and 1906. Living trichinæ were found in 1.41% and in addition 1.16% contained trichina-like bodies or disintegrating trichinæ. In other words, 1 out of every 79 hogs was infected, or, if dead and trichina-like bodies are included, 1 out of every 39 hogs. Blumer[6a] states that 6% of American hogs have the disease, and Hall[16b] estimates the incidence at 1 to 2%. The parasite is most prevalent among garbage-fed hogs, between 4 and 5% being infected. The fallacy in the idea that, since Federal examination of millions of hogs showed a trichina incidence of only about 2%, the danger of infection is slight, is pointed out by Riley and Scheifley.[30] The average found in millions of examinations is not applicable to any given community since there might be practically no infected animals or almost any ratio might exist, depending on local or temporary conditions.

Course and Diagnosis. Unlike many infectious diseases, the amount of infected material ingested determines the severity of the symptoms in trichinosis. If the meat is only slightly infested, large quantities must be eaten to produce ill effects, and the meat must be heavily infected if severe trichinosis is to follow the ingestion of small amounts. This fact was first pointed out by Ransom.[28]

A note written on December 22, 1897, by Osler on a case of trichinosis in the Johns Hopkins Hospital, reported by Thayer,[24] describes the classic picture: "The face and eyelids are puffy, the face suffused and red, the tongue clean, the arms and hands much suffused—the latter being cyanotic, they are closed with difficulty as they are so stiff. The feet and arms are considerably swollen, particularly over the backs and wrists where they are puffy. The swelling of the arms is actually in the muscles which are sore; there is distinct soreness of the trunk. The feet are livid, the legs still and cold, the muscles of the calves are not particularly swollen, not very tender."

According to Ransom,[28] trichinosis is difficult to diagnose and an important characteristic of the disease, whether mild or severe, is the lack of regularity in its course. Hall[16a] states that no adequate clinical picture of trichinosis is available and that the effects produced by the worm will be conditioned by the following factors: 1, the number of worms present; 2, the size of the patient; 3, the tissues invaded; and 4, the physical condition, resistance and concomitant pathologic conditions in the individual attacked by the parasite. When considered from this point of view trichinosis becomes a highly complicated and little studied disease. The onset varies considerably as pointed out by Blumer.[6b] In one group of patients, gastro-intestinal symptoms appear within a few hours after the ingestion of infected meat and may continue until manifestations of the invasion of the body by the young parasites appear. In another group of patients there is no immediate effect, but after an interval of 6 to 14 days symptoms associated with the dissemination of the larvæ through the blood stream occur. This difference in the onset has not been explained, but it is possible that it is due to variation in dosage.

When the infection is severe the disease can be divided into three stages:

1. The stage of intestinal infestation—the symptoms are referable to the gastro-intestinal tract and usually begin within a few hours after ingestion of infected meat. Nausea, vomiting, diarrhea and abdominal cramps are usually present and the temperature rises gradually. There is considerable prostration with pain and stiffness in the muscles.

2. The stage of dissemination—the embryos usually enter the blood and lymph stream about the end of the first week. Edema of the face and eyelids is common at this period, but its mechanism is not clearly understood. Fever and profuse sweating are often present. The muscles, especially those of respiration, mastication and the eye, are painful and their function is disturbed. Bronchitis may be present and the patient is inclined to be apathetic and prostrated.

3. The stage of encystment—about the end of the sixth week the

TABLE 1.—CLINICAL DATA ON 23 CASES OF TRICHINOSIS.

Case.	Date.	Age.	Sex.	Presenting complaints.	History.	Maximum W.B.C. in thous.	Maximum eosinoph., %.	Fever.	Muscle tenderness.	Edema.	Biopsy.	Outcome.
1	10/ 5/31	22	M	Malaise, aching, fever, sore throat	Unessential	27.2	57	Intermittent	No	No	Inflammatory infiltration, eosinophils, no parasites	Recovered.
2	11/13/31	17	F	Pain in extremities, edema, abdominal tenderness	Ingestion of raw pork 6 wks. before	16.4	4	Remittent	Yes	Legs, arms, eyelids	Myositis, trichinæ at autopsy	Died 7 days after admission.
3	3/28/32	37	F	Edema, headache, vomiting	Unessential	8.1	24	Intermittent	Yes	Legs, eyelids	Myositis, no parasites	Recovered.
4	7/13/32	20	F	Muscle pain, malaise, anorexia, weakness, fever	Ingestion of raw pork and beef	16.1	32	Remittent	Yes	Eyelids	Not permitted	Recovered.
5	7/13/32	28	M	Weakness, daily fever	Ingestion of raw pork and beef	25.8	12	Intermittent	No	No	Inflammatory infiltration between muscle fibers, no parasites	Recovered.
6	8/19/32	18	M	Edema	Ingestion of bologna	14.3	11	None	No	Eyelids and face	No parasites	Recovered.
7	5/ 7/34	23	M	Backache, edema	Unessential	19.3	45	None	Yes	Ankles, arms, face	Myositis, lymphatic infiltration	Recovered.
8	7/28/34	26	F	Edema, headache, fever	Unessential	10.5	51	Intermittent	No	Eyelids and face	Inflammatory reaction, trichinæ found	Recovered.
9	6/29/34	23	M	Muscle pain, sore throat, vomiting	Ingestion of pork	17.7	30	Intermittent	Yes	Eyelids	No parasites	Recovered.
10	8/17/34	23	F	Pain in legs and abdomen	Unessential	17.9	59	Slight	Yes	No	No parasites	Recovered.

10	8/17/34	23	F	Pain in legs and abdomen	Unessential	17.9	59	Slight	Yes	No	No parasites	Recovered.
11	11/ 4/34	20	F	Edema, muscle pain, headache	Ingestion of raw pork	13.0	23	Remittent	Yes	Eyelids	Inflammatory infiltration, eosinophils, no parasites	Recovered.
12	9/17/35	19	M	Edema, muscle pain	Ingestion of cooked sausage	24.1	51	Remittent	Yes	Eyelids	Inflammatory infiltration, eosinophils	Recovered.
13	7/18/36	28	M	Diarrhea, abdominal pain	Ingestion of poorly cooked pork	14.0	47	No	No	No	Inflammatory reaction, no parasites	Recovered.
14	12/30/36	57	M	Vomiting, diarrhea, weight loss	Unessential	14.0	52	No	No	No	No parasites	Recovered.
15	1/ 7/37	34	M	Diarrhea, malaise, weakness	Ingestion of raw pork	19.3	16	Intermittent	Yes	Yes	Trichinæ present	Recovered.
16	8/12/37	22	F	Diarrhea, vomiting, headache	Ingestion of raw pork	28.0	73	Intermittent	No	Eyelids	Inflammatory infiltration, eosinophils	Recovered.
17	2/27/37	37	M	Diarrhea, malaise, insomnia	Ingestion of raw pork	17.3	37	Slight	No	No	Inflammatory reaction, no parasites	Recovered.
18	10/ 2/37	30	F	Muscle pain, edema	Ingestion of raw pork	5.3	11	Remittent	Yes	Eyelids	Trichinæ present	Recovered.
19	4/18/38	21	M	Chills, malaise, pain in legs	Ingestion of rare pork	12.2	37	Slight	Yes	Face	Not permitted	Recovered.
20	5/ 3/38	50	F	Chills, vomiting, pain in legs and abdomen	Ingestion of poorly cooked pork	9.3	38	Slight	Yes	No	Focal myositis, inflammatory infiltration, eosinophils, no parasites	Recovered
21	6/15/32	43	F	Vomiting, delirium, meningeal symptoms	Ingestion of pork	21.8	12	Yes	Yes	Yes	Diagnosis of trichinosis at autopsy	Died.
22	7/10/33	40	M	Vomiting, diarrhea	Ingestion of bologna	18.0	38	Yes	Yes	Yes	Trichinæ present	Recovered.
23	10/25/30	32	M	Edema	Ingestion of hamburgers	14.5	21	Slight	Yes	Yes	Trichinæ present	Recovered.

embryos become encysted in the muscles. At this time the symptoms become less severe and the temperature gradually returns to normal. The patient is anemic and emaciated and the edema subsides gradually. This late edema is probably due to renal involvement and cachexia. The muscular pains and weakness may persist for several months.

Early in the disease there is a marked increase in the percentage of red blood cells and a moderate increase in the percentage of hemoglobin. Later anemia develops, the number of red cells falling as low as 2,500,000 per c.mm. and the hemoglobin as low as 45%. Leukocytosis, usually about 25,000 white blood cells per c.mm., is often found. When the embryos invade the tissues, the eosinophils increase to 15 to 30% or more; in 1 of our cases an eosinophilia of 73% was found.

Beside these manifestations which constitute the clinical picture of the typical case of trichinosis, other less common signs and symptoms have been reported. General fatigue, generalized edema, cough, headache, chills, furuncles and hoarseness were found in the cases of McDonald and Waddell.[23] In the more severe cases, Cheney[9] and Carlson[7] noted a marked hypotension. The heart in patients with trichinosis has been studied and both clinical and electrocardiographic evidences of various degrees of temporary myocardial involvement were reported by Spink,[32a] Cushing[11] and Beecher and Amidon.[5] The spleen may be palpable and this condition may persist for several months according to Reifenstein, Allen and Allen.[29]

Conner[10] describes some of the deviations from the usual clinical picture of the disease that may be seen. Some cases may run an afebrile course. The characteristic eosinophilia may be lacking throughout the whole course or for the first few weeks of the disease. Occasionally a positive Widal reaction makes the differentiation from typhoid fever difficult. A few cases resemble acute nephritis or meningitis, and at times there may be alarming throat symptoms or a frontal sinusitis. In a small percentage of cases epigastric pain is the chief or only symptom.

Frequently trichinosis is complicated by the involvement of various organs. Ocular manifestations, such as chemosis, exophthalmos, conjunctival ecchymosis, mydriasis and retinal hemorrhage were described by Carter.[8] Neurologic and mental symptoms were present in some of the patients reported by Merritt and Rosenbaum[24] and Most and Abeles.[26] Herrick[19] and Kilduffe, Barbash and Merendino[21] noted instances of femoral phlebitis and femoral thrombosis, and pneumonia occurred in several cases cited by McDonald and Waddell.[23]

Special Diagnostic Aids. In 1928, Bachman demonstrated precipitins in the serum of individuals infected with the Trichinella spiralis[4a] and also noted that a specific local skin reaction followed the injection of trichinella protein.[4b,c] He concluded that as an aid

in diagnosis the skin reaction is easy and more practical than the precipitin test, since typical skin reactions appear 20 to 30 days before precipitins are demonstrable in the blood stream. Some investigators (McCoy, Miller and Friedlander,[22] Kilduffe,[20] Spink and Augustine,[33] Heathman[18]) have found that these special diagnostic aids are not entirely reliable and that the presence of eosinophilia and muscle biopsy are more practical and valuable. However, in ruling out trichinosis and detecting mild, sporadic and atypical cases the Bachman skin test may be a valuable aid (Friedlander,[13] Spink and Augustine,[33] Spink,[32b] Augustine[3]). Hall[15] reported 8 cases of trichinosis all of which gave a positive reaction to the intradermal, and Blumer,[6a] Vener and Stevens[35] and Schapiro, Crosby and Sickler[31] have all found that positive reaction occurs early in the disease. Our experience has been that the blood counts, muscle biopsy and skin test are all of value as diagnostic aids.

Summary. The following points summarize the safeguards that we believe should be used for the control of trichinosis:

1. Individuals by their food habits expose themselves to the disease and the public should, therefore, be informed of the danger of trichinosis. They should be taught emphatically that insufficiently cooked food containing any pork is dangerous. The consumer must be brought to realize that he is, in the last analysis, responsible for getting trichinosis.

2. As Pote[27] points out, the least likely source of the parasite is pork products prepared under Federal and adequate municipal supervision. We agree with his theory that it is the unprocessed pork products, especially summer sausage, prepared without supervision in small local slaughter houses or on farms, that frequently cause trichinosis. The jurisdiction of public health organizations should be made to include these sources of pork products.

3. The packer and swine grower, as pointed out by Hall,[16c] have it within their power and should be given the responsibility of setting up safeguards against the consumption of infested pork, particularly by excluding the hog which is fed uncooked pork scraps.

4. The medical profession should keep in mind the possibility of the disease when unusual cases of "flu" or "grippe" do not respond readily to treatment. If the physician suspects the disease the diagnosis usually will be made readily. All cases should be reported to the Health Department and attempts made to discover the source of the parasite.

Finally, we should like to emphasize that while the immediate responsibility for contracting trichinosis rests upon the consumer, the United States Public Health service should assume the chief rôle in protecting the public from this disease.

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STUDIES WITH THE AGAR CUP-PLATE METHOD.

II. The Effect of Blood on Mercury Antiseptics.

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Although mercury compounds yield remarkably high phenol coefficients (Thomas;[13] Birkhaug[3b]), it is well known that they are influenced markedly by protein (Abbott;[1] Smith, Czarnetzky and Mudd;[12] and others). Therefore a high phenol coefficient for a given mercurial does not presuppose comparable effectiveness in the presence of blood. On the contrary, as will be shown, such data may be meaningless and even misleading.

The high antiseptic values of organic mercurials has stimulated the study of these substances in the intravenous treatment of experimental and clinical bacteremias. However, such investigations have yielded conflicting data. For example, Raiziss, Severac, and Moetsch[9] reported favorable results when metaphen was used for the treatment of artificial *Staph. aureus* bacteremia in rabbits.

Similarly, Lambert[6] obtained encouraging results with the use of merthiolate in clinical tuberculosis, and Barthelme[2] found a lowered mortality rate in tularemia when patients were treated intravenously with metaphen. On the other hand, Douglas and Birk-

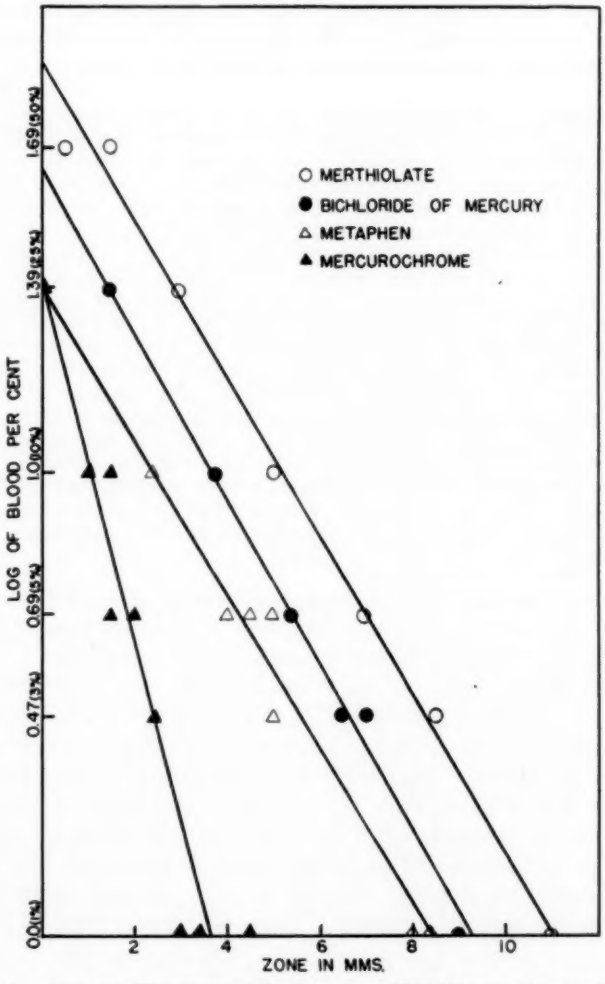

CHART 1.—Zone in millimeters produced by 1 to 1000 dilution of mercurial antiseptics in varying concentrations of blood. As the blood concentration increases, the size of the zone of inhibition for *Staph. aureus* decreases. The relative potency of the four mercurials is evident.

haug[5] found that metaphen accelerated a fatal issue in experimental bacteremia with pneumococcus Type I, and Cummins,[4] Mallick, Ali and Singh[7] failed to obtain favorable results in the treatment of clinical tuberculosis with intravenous merthiolate medication.

The discrepancy between the known potency of mercurials as determined by the phenol coefficient method and their disputed potency *in vivo* appears to be associated with the blood factor (Simmons;[11] Birkhaug[3a]). Therefore, it is clear that the limits of activity of mercury compounds in the presence of whole blood should be determined before the use of these substances receives further serious consideration in intravenous chemotherapy. Our experiment was planned to study such limiting values.

Technique. A standardized agar cup-plate method (Rose and Miller[10]) was employed. The test organism was a stock culture of *Staph. aureus* (WP3 strain), grown in broth for 18 to 20 hours, standardized to 1 billion organisms per cc., and used in the ratio of 0.1 cc. to each 30 cc. volume of agar. The basal medium was 3% beef extract agar, pH 6.8; this was mixed with equal volumes of either defibrinated horse blood* or horse blood-saline dilutions to make an agar concentration of 1.5%. Blood agar plates with the following blood content were prepared: 1%, 3%, 5%, 10%, 25%, and 50%. When the medium hardened, cups were made by removing discs of agar 1.5 cm. in diameter. Aqueous 1 to 1000 dilutions of mercurochrome, metaphen, merthiolate,† and mercury bichloride were prepared. A 1 to 500 aqueous solution of metaphen was used as a stock solution because the powder was not available. All dilutions of the other mercurials were made by dissolving accurately weighed powder in double distilled water. Each antiseptic in 0.2 cc. amounts was placed in cups of the various blood agar media. After 24 hours' incubation at 37° C., the zone of inhibition of bacterial growth was measured and recorded in millimeters. The log of the blood concentration (ordinate) was plotted against the zone size (abscissa). The results of the experiment are shown in Chart 1.

The four antiseptics used showed a decreasing effectiveness in the following order: merthiolate, mercury bichloride, metaphen, mercurochrome. The variation in antibacterial properties is evident from the figure. As the blood concentration rose all compounds showed a diminishing activity. At the 25% blood level, metaphen and mercurochrome were approaching a zero value. Similarly, the activity of mercury bichloride was extinguished somewhat below the 50% blood level, while the antiseptic properties of merthiolate were still in evidence when half the medium consisted of blood. The zone of inhibition of bacterial growth at any blood level may be determined‡ for a given dilution of each mercury antiseptic by applying the general formula ("Law of Organic Growth"), $C = Ke^{-mz}$.

C, concentration of blood in per cent; K, a constant for each antiseptic having the value of C when the zone is zero; e, base Napierian log; m, coefficient of inactivation of the antiseptic; z, zone in millimeters.

* Obtained through the courtesy of the Mulford Laboratories, Sharp and Dohme, Glenolden, Pa.

† Obtained through the kindness of the Eli Lilly Co., Indianapolis, Indiana.

‡ We are indebted to Mr. Charles Robb for his invaluable help in the mathematical analysis of the data.

The application of the formula may be illustrated as follows: Let us assume that the zone size in 2% blood is desired for a 1 to 1000 dilution of mercurochrome. For mercurochrome, K = 24.55; m = 0.875.

$$\begin{aligned} C &= Ke^{-mz} \\ &= 24.55e^{-0.875z} \\ \log C &= \log 24.55 - (0.875)z \log e \\ &= \log 24.55 - (0.875)\,(0.4343)z \\ \text{or } z &= \frac{\log 24.55 - \log C}{0.38} \end{aligned}$$

To determine z when the blood concentration is 2%:

$$\begin{aligned} z_2 &= \frac{\log 24.55 - \log 2}{0.38} \\ &= \frac{1.390 - 0.301}{0.38} \\ &= 2.8 \text{ mm.} \end{aligned}$$

Comment. Our experiments show that organic and inorganic mercury compounds follow a similar pattern of inactivation by blood. The fact that a single mathematical formula applied equally well in all cases lends support to the validity of the data.

No attempt was made to establish the usefulness of mercurials on intact skin surfaces. However, if the skin is broken and blood is present in a wound, the antiseptic value of these substances may be questioned on the basis of our data.

The usual procedure for testing antiseptic potency has been the phenol coefficient technique. However, the data obtained by this method have been inferentially misleading and have encouraged intravenous mercurial chemotherapy. It is clear that mercury compounds should be appraised under circumstances which simulate their application in clinical conditions. The implications concerning the intravenous use of mercurials is obvious.

Studies dealing with the influence of agar *per se* on zone size (Miller and Rose[8a]) and the correlation of agar cup-plate data with an antiseptic-dilution testing procedure (Miller and Rose[8b]) will be presented in separate communications.

Summary and Conclusions. Using a standardized agar cup-plate technique, 4 mercury antiseptics (mercury bichloride, mercurochrome, metaphen, and merthiolate) were studied in horse blood-agar mixtures. The blood content of the test media varied from 1 to 50%. The test organism was *Staph. aureus* (WP3 strain).

All 4 mercury compounds showed a diminishing antiseptic effectiveness as the blood concentration was increased. The inactivation of the mercurials was expressed by the general formula, $C = Ke^{-mz}$. The limits of activity of the mercurials indicated that these substances had no antiseptic properties at certain critical blood levels. The *in vitro* experimental data suggested that mercury compounds could have no value in intravenous therapy.

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A NEW DIAGNOSTIC TEST (GALACTOSE) FOR THYROID DISEASE.*†

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Two years ago we reported finding a consistent reduction of "tolerance" to galactose in a group of 26 patients with hyperthyroidism.[2] This lead was followed by an experimental investigation to discover the cause of this phenomenon, and a clinical study of a larger series of patients to determine whether the galactose tolerance test would be useful in the diagnosis of disorders of the thyroid gland.

This investigation[1] showed that in rats experimental hyperthyroidism caused increased intestinal absorption of sugars, which probably is responsible for the abnormally high values obtained for galactose and for glucose in the blood of patients with hyperthyroidism following tolerance tests with these sugars. Thyroidectomy in rats was found to result in decreased absorption of glucose, which offered a more rational explanation than increased utilization of sugar for the low glucose tolerance curves characteristic of patients with myxedema. Evidence was obtained to indicate that increased absorption of sugars was probably due to stimulation of phosphorylation in the intestinal mucosa by thyroxin.

* Read before the Annual Meeting of the Association for the Study of Goiter at Cincinnati, May 22–24, 1939.

† Aided by a grant from the Christine Breon Fund for Medical Research.

In the present paper we are reporting our clinical experiences with the galactose tolerance test in 130 patients with hyperthyroidism, 121 individuals without hyperactivity of the thyroid gland and 7 patients with myxedema.

Technique of the Test. Forty grams of galactose (Pfanstiehl) dissolved in 400 cc. of water and flavored with lemon juice were administered by mouth after the patient had fasted over night. Specimens of blood were obtained from the cubital vein before, and 5, 15, 30, 60 and 120 minutes* after administration of galactose.

The glucose fraction of the blood was removed by fermentaton with ordinary yeast according to the method of Somogyi[5] as modified by Raymond and Blanco.[3]

Preparation of Yeast. A weighed amount of fresh commercial yeast (Fleishman's) is suspended in 5 to 10 parts of water, centrifuged, and decanted. This is repeated until the supernatant liquid is clear and colorless and gives no reduction test (6 to 7 washings). The yeast is then suspended in 10 parts of water. In this condition, it will keep well in a refrigerator for about 2 weeks.

Use of the Hagedorn-Jensen Method. Two-tenths cubic centimeters of blood is collected into an accurately calibrated pipette (we use 0.2 ml. in 0.001 ml. Kahn pipette No. 37036); and transferred to 2.3 cc. of distilled water, rinsing the pipette once or twice with the solution. One cubic centimeter of the 10% yeast suspension is then added, the contents mixed by tapping the tube against the hand, and after 4 or 5 minutes 0.5 cc. of tungstic acid solution (prepared freshly by mixing equal volumes of 10% Na_2WoO and 2/3 N H_2SO_4) is added. The test tube is covered with the thumb and the contents are mixed quickly by inverting. After standing a few minutes, the mixture is centrifuged at high speed. One cubic centimeter of the clear filtrate is used for titration.

Use of the Folin-Wu Method. The yeast is washed in the usual manner and a 20% yeast suspension is prepared. To 2 cc. of oxalated blood, 14 cc. of the yeast suspension and 4 cc. of the tungstic acid solution are added. This mixture is filtered, and 2 cc. portions of the filtrate are used for sugar determinations.

The figure for non-fermentable reducing substances in the fasting blood is subtracted from the corresponding figure in the remaining specimens to obtain the galactose content of the blood titrated as glucose. In order to obtain the true value for galactose, which has a lower reducing power than glucose, 24% must be added to the last figure.

Galactose is used in this test because, while having a rate of absorption very similar to that of glucose, it has the advantage that, unlike glucose, it can be identified in the blood.

Results. NORMAL SUBJECTS AND PATIENTS WITHOUT HYPERTHYROIDISM. In 10 normal volunteers, blood galactose curves for 2-hour periods were obtained. The composite curve of the results is shown in Figure 1. It reaches a maximum of 19 mg.% at the end of 60 minutes. The peaks of the individual curves ranged between 13 and 31 mg. per 100 cc. and were found to occur in approximately one-half the patients in the 30-minute specimen, and in the other one-half in the 60-minute specimen. In the 5-minute specimen,

* At present we consider the 15- and 120-minute specimens unnecessary, and the 5-minute specimen relatively unimportant except when the test is repeated on account of borderline results.

galactose in very small amounts (up to 4 mg. per 100 cc.) was present in only 2 instances. At the end of 2 hours, galactose in small amounts was present in the blood only exceptionally.

The galactose tolerance test was used also in 87 patients suffering from diseases other than hyperthyroidism or myxedema (Fig. 1).

Almost all of the patients with the following diseases were found to have normal or low galactose tolerance curves: diabetes mellitus, Addison's disease, acromegaly (in the late stages), Cushing's disease, osteitis fibrosa cystica, spontaneous hypoglycemia, congestive heart

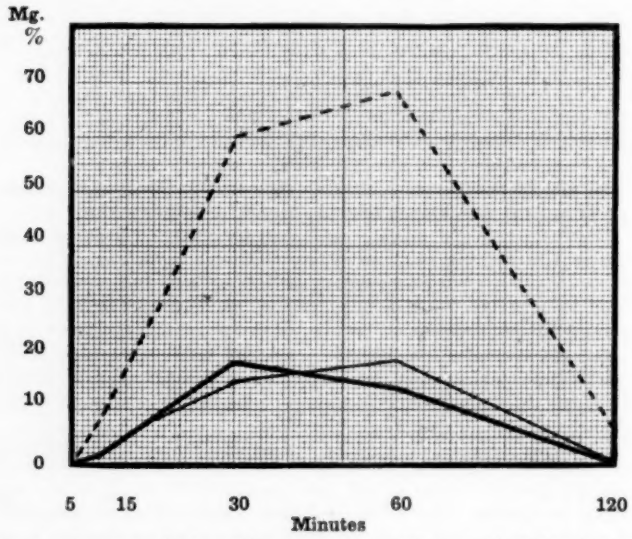

FIG. 1.—Average galactose tolerance curves of 10 normal individuals (narrow line), 87 patients without hyperthyroidism or myxedema (wide line), and 130 patients with clinical hyperthyroidism (broken line).

failure, vascular hypertension, secondary anemia, peptic ulcer, carcinoma of the stomach, idiopathic ulcerative colitis, rheumatoid arthritis, osteosarcoma, syphilis, pellagra, anorexia nervosa, diarrhea, intestinal hypermotility and menopause.

In a single case of each of the following conditions there was an abnormally high galactose curve in the blood: diabetes mellitus,* congenital syphilis with chronic mastitis, hyperinsulinism, intractable gastric ulcer of long standing, intestinal hypermotility, Cushing's disease and prolonged fever of unknown origin.

The distribution of the peaks of the galactose curve in normal subjects and in patients without hyperthyroidism is shown in Figure 2.

HYPERTHYROIDISM. The galactose tolerance test was given to 130 patients with clinical symptoms and signs of hyperthyroidism:

* One case out of 15.

in all cases the diagnosis was made by the Thyroid Committee of the University of California Hospital.* In difficult cases, the final diagnosis was made only after periods of observation and treatment that lasted for weeks or months.

The composite curve of the galactose tolerance test in our cases of hyperthyroidism is given in Figure 1. It reaches a maximum of 68 mg. per 100 cc. in the 60-minute specimens. The peaks of individual curves varied from 25 to 152 mg. per 100 cc. (Fig. 2). As in the case

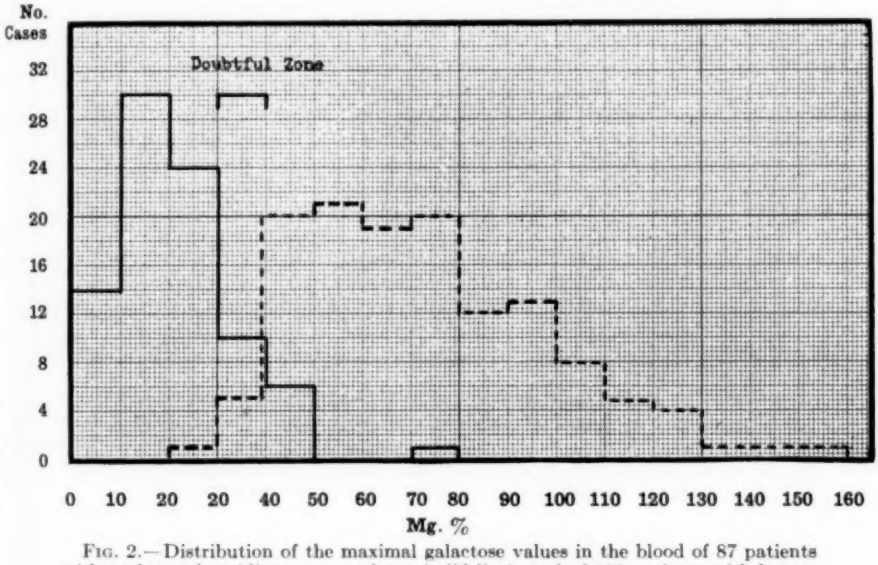

FIG. 2.—Distribution of the maximal galactose values in the blood of 87 patients without hyperthyroidism or myxedema (solid line), and of 130 patients with hyperthyroidism (broken line).

of normal subjects, about one-half of the peaks occurred in the 30- and one-half in the 60-minute specimens. Most patients with hyperthyroidism had some galactose in the 5-minute specimen of blood, and in one-half of the cases the amount was between 9 and 20 mg. per 100 cc. At the end of 2 hours, galactose was seldom found in the blood. The distribution of the readings of the basal metabolic rates in patients of this group is given in Figure 3. When several basal metabolic rate estimations were obtained on the same patient, the test most nearly concurrent with the galactose tolerance test was chosen.

EFFECTS OF THYROIDECTOMY. In 22 cases, the galactose tolerance test was repeated after thyroidectomy. (See Fig. 4.) In these 22 cases, 18 of the curves returned to normal range after operation, 2 were lower than the corresponding preoperative curve

* This committee, which holds weekly conferences, consists of a group of internists and surgeons and one radiologist, who are making a special study of diseases of the thyroid gland.

but remained within the pathologic range, and 2 were unchanged at the time of discharge from the hospital. Of these last 2 patients, 1 had had a preoperative basal metabolic rate of 21%+, which was reduced by the operation only to 14%+.

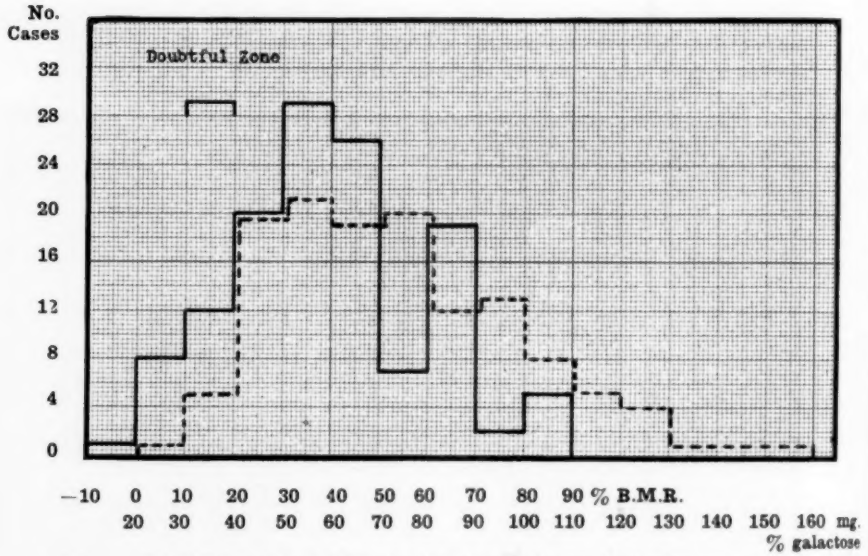

FIG. 3.—Distribution of the B.M.R. (solid line) and the maximal galactose values in the blood of 130 patients with clinical hyperthyroidism (broken line).

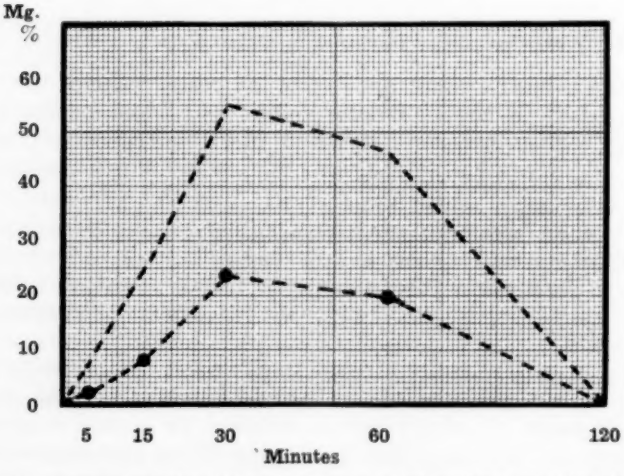

FIG. 4.—Average galactose tolerance curves of 22 patients who had thyroidectomy for hyperthyroidism before (broken line) and after operation (broken line with discs).

Myxedema. Six patients with spontaneous myxedema and basal metabolic rates ranging between 19 and 33%− had galactose tolerance curves with peaks from 3 to 11 mg. per 100 cc. One patient with postoperative myxedema and a basal metabolic rate of 27%− was found to have no galactose in the blood during the galactose tolerance test.

Conditions Clinically Simulating Hyperthyroidism With Normal Galactose Tolerance. In this classification, we were interested in three conditions:

1. *Anxiety With Hyperventilation.* The basal metabolic rates of 8 patients in this group were elevated to between 22 and 36%+. The maximum galactose content of their blood was between 18 and 35 mg. per 100 cc.. Of 13 other patients with anxiety states that clinically suggested hyperthyroidism but had basal metabolic rates under 16%+, 11 had peaks of the galactose curve between 9 and 30 mg. per 100 cc., 2 had peaks of 46 and 49 mg. per 100 cc. respectively.

2. *Diseases of the Heart With Increased Basal Metabolic Rate Estimations.* Four patients with cardiac lesions who had basal metabolic rates between 24 and 32%+ were referred to us with the diagnosis of hyperthyroidism. The peaks of the galactose curves in these patients were between 9 and 36 mg. per 100 cc.

3. *Diseases of the Thyroid Gland Other Than Hyperthyroidism.* Three patients with non-toxic adenoma of the thyroid had a maximal galactose content of the blood between 11 and 24 mg. per 100 cc. Two patients with Hashimoto's struma and basal metabolic rates of 9%+ and 44%+ had the peak of the galactose curve at 4 and and 33 mg. per 100 cc. respectively.

Diseases Other Than Hyperthyroidism With Abnormal Galactose Tolerance. 1. *Diseases of the Liver.* In 11 patients with deep jaundice due to parenchymatous diseases of the liver, we found a true impairment of tolerance to galactose as shown by the rate of disappearance of galactose injected intravenously. Therefore high blood galactose curves after oral tolerance tests with this sugar may be expected in cases of marked functional insufficiency of the liver. This actually occurred in several patients in whom the oral galactose tolerance test was done to test this assumption. A separate report will deal with our work on galactose tolerance in patients with hepatic diseases.

2. *Paget's Disease.* Fifteen of 18 patients with this condition were found to have higher than normal galactose tolerance curves, apparently due to increased intestinal absorption. Our data on patients with Paget's disease will be published in another paper.

The results of galactose tolerance tests on patients with hepatic involvement and with Paget's disease are not included in Figures 1 and 2.

Repetition of the Galactose Tolerance Test. In 10 patients with or without hyperthyroidism, the galactose tolerance test was

repeated in order to determine the degree of uniformity of the resulting curves. In 1 case the difference between the peaks of successive tests was 40 mg.,* in another 21 mg., in 2 cases 9 mg. and in 6, 3 mg. or less.

Intravenous Administration of Galactose. It was important to determine whether inability to utilize galactose due to hepatic insufficiency played a part in producing the abnormally high galactose tolerance curves in our patients with hyperthyroidism. For this purpose, 10 patients with hyperthyroidism, who had very high maximal blood galactose values averaging 87 mg. per 100 cc. after the oral test, were given 40 gm. of galactose intravenously. Then the rate of disappearance of galactose from the blood was determined and compared with that of 10 normal subjects and of 11 patients with parenchymatous diseases of the liver. This comparison showed that in patients with hyperthyroidism tested by the intravenous method, the utilization of galactose was within normal limits.

One patient with myxedema and a maximal blood galactose value of 3 mg. per 100 cc. had an abnormally prolonged galactose curve in the blood following intravenous administration of this sugar.

Comment. From the distribution of the peaks of the galactose curves in subjects with and without hyperthyroidism (Fig. 2), it is seen that in round figures, the normal range for the maximal concentration of galactose in the blood under the conditions of our test is between 10 and 30 mg. per 100 cc. A peak between 30 and 40 mg. per 100 cc. in the galactose tolerance curve lies in the doubtful range. Values exceeding 40 mg. per 100 cc. for galactose in the blood are abnormal. So also are maximal values below 10 mg. per 100 cc. Repetition of the test in the same person usually gives fairly uniform maximal values. When these criteria were applied to our group of 130 patients with hyperthyroidism, the galactose tolerance test was positive in 124 cases, doubtful in 5 and negative in 1. In a group of 87 patients with conditions other than hyperthyroidism, proven hepatic insufficiency, or Paget's disease, the outcome of the test was positive in 7 instances, doubtful in 10 and negative in 70. In 4 of the 7 patients with a positive outcome of the test, involvement of the liver was suspected. The galactose tolerance curve was abnormally low in 6 out of 7 patients with myxedema, and barely within the lower limit of normal in 1.

In the normal state, little if any galactose is found in the 5-minute specimen. In hyperthyroidism, increased intestinal absorption of sugars is expressed not only by a high peak of the galactose curve but also, in about one-half of the cases, by a premature appearance in the blood of considerable amounts of galactose. Therefore the finding of 10 mg. per 100 cc. or more of galactose in the 5-minute

* This was in a patient with hyperthyroidism who in 2 tests had peaks of 90 and 50 mg. per 100 cc. but was treated with Lugol's solution between the tests.

specimen is an additional criterion in favor of the diagnosis of hyperthyroidism. This criterion is naturally most useful in instances where the peak of the galactose curve is in the doubtful zone.

A comparison in hyperthyroid patients of the results of the galactose tolerance test with their basal metabolic rates* shows that no close quantitative correlation between the two exists, although usually patients with very high basal metabolic rates also have very high values for galactose in the blood. The reason for such a lack of correlation is not clear. Administration of a standard amount of galactose regardless of body weight probably does not explain it, because 40 gm. galactose exceed the capacity for intestinal absorption during the period of the test. Moreover, the correlation was no greater when the two tests were compared in groups of patients classified according to weight. Probably the best explanation is that elevation of the basal metabolic rate and stimulation of absorption are relatively independent effects of thyroxin which occur to varying degree in different patients. This possibility is also suggested by our experimental data[1] showing that an increase in basal metabolism produced by administration of alpha-dinitrophenol or by overheating had no stimulating effect on the intestinal absorption of glucose.

The well-known occurrence of true hyperthyroidism without elevation of the basal metabolic rate is also in favor of this conception. An interesting example of this condition was encountered during the present study. Mr. H., aged 42, entered with a clinical picture typical of Graves' disease, but by repeated estimations his basal metabolic rate was found to be between 5 and 10%−. A galactose tolerance test showed 10 mg. per 100 cc. of this sugar in the 5-minute specimen and 55 mg. per 100 cc. at the peak of the curve. After much debate in the Thyroid Committee, the patient was subjected to a subtotal thyroidectomy. Microscopic examination of the thyroid gland showed characteristic hyperplasia. Following operation the patient lost his symptoms and signs indicative of hyperthyroidism. A repetition of the galactose tolerance test showed no galactose in the 5-minute specimen and a maximum of 21 mg. per 100 cc. of galactose in the blood. Several basal metabolic rate determinations averaged only about 5% lower than before operation.

In comparing the clinical value of the galactose tolerance test with that of the basal metabolic rate, it appears that among patients with definite clinical hyperthyroidism doubtful results† are encountered somewhat less frequently with the galactose tolerance test (Fig. 3). This is probably due to the fact that the intestinal absorption of sugars is more sensitive to the influence of a small

* In this study, the basal metabolic rates were estimated for 8-minute periods by the indirect method with the Benedict-Roth apparatus and the use of the nose clip.

† Defined for the galactose tolerance test as under 40 mg. per 100 cc. and for the basal metabolic rate as under 10% plus.

excess of circulating thyroxin than is the basal metabolism. At least Russell[4] in a study undertaken at our suggestion found that in rats after hypophysectomy, which depresses both the absorption of sugars and the basal metabolic rate due to lack of the thyrotropic hormone of the anterior lobe of the hypophysis, administration of thyroxin in amounts sufficient to restore normal intestinal absorption raised the basal metabolic rate only half-way back to the original normal level.

When the basal metabolic rate is found to be definitely elevated in patients with hyperthyroidism, that test seems to offer a more accurate index of the degree of hyperthyroidism than does the galactose tolerance test.

In the differential diagnosis of hyperthyroidism, the galactose tolerance test is useful in cases of low grade hyperthyroidism, especially when the B.M.R. is less than 20%+. and in patients without hyperthyroidism who have an abnormally elevated B.M.R. Fifteen of our 130 patients with hyperthyroidism belong in the first classification, and in all of these the galactose test was positive. It is of interest that the proportion of patients with low grade hyperthyroidism in our series was almost identical with that recently reported by Young and Krantz.[6] In the second classification were 8 patients suffering from anxiety states with hyperventilation, and 4 patients with obscure cardiac dyspnea. The B.M.R. in these 12 patients ranged from 22 to 36%+, whereas the galactose test was negative in 10 cases and doubtful in 2*.

In 5 patients with non-toxic adenoma of the thyroid or with Hashimoto's struma, the results of the galactose tolerance test corresponded to estimations of the B.M.R.

Advanced hepatic insufficiency and Paget's disease interfere with the use of the galactose tolerance test for the diagnosis of hyperthyroidism. However, since in hepatic insufficiency there is a true impairment of utilization of galactose whereas in hyperthyroidism the high galactose tolerance curve is due to increased intestinal absorption, this difference can be brought out by administering the galactose again at a later date intravenously and following the rate of its disappearance from the blood. In this connection it must be remembered that several authors, including ourselves, have found hepatic insufficiency as indicated by various liver function tests to be present in some patients with severe hyperthyroidism.[2]

Intravenous administration of galactose to patients with hyperthyroidism showed that, as a rule, utilization of galactose in this condition is normal. This finding represents a clinical confirmation of our experimental work on animals indicating increased intestinal absorption in hyperthyroidism.

As a rule, subtotal thyroidectomy promptly restores the blood

* A patient with myeloid leukemia and a B.M.R. of ½%+ also had a normal galactose test.

galactose curve to normal, but occasionally it fails to accomplish this. Unfortunately, we were unable to repeat the galactose tolerance test on our 2 patients in whom the galactose curve had remained unchanged after operation. A possible explanation of these unchanged curves is that the amount of thyroid tissue removed in these cases at operation may have been insufficient. This is suggested by the great sensitivity of intestinal absorption to thyroxin and by the fact that in 1 of the 2 cases thyroidectomy also produced little change in the basal metabolic rate.

Our experience with 7 patients who had myxedema suggests that the galactose tolerance test could be used also in the diagnosis of doubtful cases of this disease. Intravenous administration of galactose to 1 patient with myxedema indicated a reduced capacity to metabolize galactose. This tends to confirm our conclusion based on experimental data that diminished intestinal absorption of sugars rather than their increased utilization accounts for the low sugar tolerance curves in myxedema.

In communities where no machine for the estimation of the basal metabolic rate is available, the galactose tolerance test can be used instead as a routine procedure for the diagnosis of hyperthyroidism, provided there are facilities for making blood sugar determinations.

Summary. 1. A new clinical test for activity of the thyroid gland based on the rate of intestinal absorption of galactose is described. It consists of oral administration of galactose followed by determinations of galactose in the blood 30 and 60 minutes later.

2. Data from 121 control subjects and 130 patients with hyperthyroidism show that the average maximal concentration of galactose in the blood of patients with hyperthyroidism was three times greater than normal and that clinically the test was comparable in reliability to estimations of the basal metabolic rate.

3. Following thyroidectomy, the galactose tolerance test was normal in almost all cases.

4. Advantages of the galactose test are that it is more sensitive than the basal metabolic rate in cases of low grade hyperthyroidism, and that its outcome is not influenced by hyperventilation in anxiety states or by cardiac dyspnea. A disadvantage of the test is that the presence of hepatic insufficiency or of Paget's disease interferes with its use for the diagnosis of thyroid disease.

5. In myxedema abnormally low galactose tolerance curves were observed, indicating that the galactose test can be used also in the diagnosis of this condition.

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PHARMACOLOGIC AND PATHOLOGIC EFFECTS OF REPEATED CONVULSANT DOSES OF METRAZOL.

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Several studies have been published recently concerning the toxic effects and pathologic tissue changes resulting from insulin shock therapy (Ferraro and Jervis[6]). Little is known as to the corresponding effects of metrazol (cardiazol, pentamethylenetetrazol). In view of the recent extensive use of metrazol, as well as insulin with metrazol, in the treatment of schizophrenia, the following investigations were undertaken.*

Recent Literature. De Morsier, Georgi and Rutishauser[16] examined 2 rabbits. The first animal, having received a total of 4.5 cc. of cardiazol in 9 injections within 1 month, had severe epileptic convulsions and was killed by bleeding. The second animal, after receiving 2 cc. of cardiazol in 1 injection, died 25 minutes later in a status epilepticus. Postmortem examinations revealed no unusual findings in the first rabbit, but a generalized hyperemia, most pronounced in the brain, in the second rabbit. The tissues appeared normal histologically.

Stender[21] examined 3 rabbits and 2 cats which had been treated with convulsant doses of cardiazol. There were 2, 25 and 31 seizures in the rabbits and 28 and 32 seizures in the cats. The findings for the most part were not significant. There were subpial hemorrhages in 2 rabbits and very small foci of softening in the brain in 1 rabbit. The author expressed the belief that these changes were of traumatic origin, caused by injuries incurred during the convulsions. In the younger cat there were some changes, similar to ischemic changes, in the nerve cells in the cortex and in the hippocampus. However, these findings were considered to be of no significant value.

* We are much indebted to Dr. Franklin G. Ebaugh, Director of the Colorado Psychopathic Hospital, for suggesting that a systematic histologic study be made of tissues from animals experimentally treated with metrazol.

Reitmann[19] studied 2 dogs: the first, weighing 8 kg., received a total amount of 38.3 cc. of cardiazol within 9 days and the second, weighing 12 kg., an amount of 68.5 cc. within 12 days. Histologically, the second dog exhibited much more severe changes. The vessels generally were dilated and in some places there was stasis. There were many hemorrhages in various places in the cortex as well as subarachnoid and intraventricular hemorrhages. No pathologic changes were revealed in the walls of vessels in the center of the hemorrhages. Several ischemic foci were present in the cortex and in the hippocampus. In places diffuse degeneration of nerve cells was associated with pericellular incrustations and dislocation of nerve cells. As a result of such studies, Reitmann emphasized that cardiazol may cause pathologic changes in the brain which obviously are due to circulatory disturbances. These changes resemble the findings of Stief and Tokay[22] in experimental insulin intoxication. Reitmann stressed the fact that he employed larger toxic doses in the experimental animals than are usually given to human beings in the metrazol treatment of schizophrenia. He was reluctant, therefore, to draw conclusions as to possible results occurring in the human brain following the use of metrazol in treatment.

Hayman and Brody[9] recently reported a fatal case, with autopsy, following metrazol therapy in schizophrenia. The cause of death in this case was considered to be the toxic effect of metrazol upon a pathologically impaired heart. The pathologic changes consisted of a preëxistent chronic endocarditis together with marked congestion of all the organs. No characteristic lesion in the brain or the other organs was found at autopsy. The authors mentioned 3 other fatal cases following metrazol therapy in schizophrenia, none of which showed any significant lesions in the brain and in all of which there was a preëxistent pathologic condition.

Strecker, Alpers, Flaherty and Hughes[23] gave metrazol to 7 monkeys in doses comparable to those usually given to human beings in the treatment of schizophrenia. Three animals did not exhibit any changes; 1 animal showed swelling and vacuolation of nerve cells in the upper cortical layers; 3 other animals showed slight subarachnoid hemorrhages. In the last-mentioned animals the cortex was remarkably well preserved. There were only a few slight changes in the cells of Betz, in the supraoptic nucleus and in the hypothalamus. But "this damage was never severe and may have no significance." The pathologic changes were seen only in animals in which the total convulsive time had been especially long (67 to 147 minutes).

Dreszer and Scholz[4] recently published the results of studies dealing with disturbances in the cerebral blood supply in generalized convulsions. They examined the brains of several cats in which convulsions had been provoked by cardiazol. The histologic work was based upon the benzidine method, which shows the distribution

of blood in the brain by the staining of the red blood cells. The authors found a definite disturbance in the capillary blood supply in the cortex in the preparoxysmal stage: there was a generalized capillary anemia together with numerous, variably-sized, circumscribed areas of more pronounced anemia. The anemia gave way to a hyperemia which began at the climax of the tonic phase of the convulsion and involved most of the vascular system during the clonic phase. The hyperemia, however, was not uniformly distributed; some anemic regions persisted at the end of the attack. A breaking-up of the blood columns into many small fragments was frequently seen in capillaries in regions originally not entirely anemic. The authors considered this to be evidence of a mild degree of capillary spasm which varied considerably throughout the anemic regions. The caliber of the large arteries seemed not to be altered. Dreszer and Scholz concluded that vasomotor disturbances play an important rôle in the causation of convulsions in experimental animals as well as in human beings.

Subsequent to convulsions induced by various means, acute destructive changes in the cortex have been described by Husler and Spatz,[12] von Braunmühl,[1] Scholz,[20] and by Neubürger.[17a] The shape of such cellular destructive changes corresponds closely to the areas of circumscribed capillary anemia occurring in the cat after convulsions induced by metrazol. Since such a marked disturbance in the blood supply follows metrazol injection, it may be assumed that pathologic changes in the nervous parenchyma are likely to result. Our histologic studies suggest this probability.

Procedure. Three dogs and 4 rabbits were given a series of convulsant doses of metrazol. The number of injections was similar to that employed clinically in the convulsive treatment of schizophrenia. The drug was given 3 times a week for a total of 14 to 18 injections. Most of the injections were given intravenously except in Rabbit 1, in which approximately one-half the injections were given intramuscularly or subcutaneously. It was frequently found necessary to increase the dose somewhat in order to maintain pronounced convulsive responses at approximately the same level. For the dogs the dosage varied from 10 to 35 mg. per kg.; for the rabbits it varied from 10 to 30 mg. per kg. These amounts are to be compared with a dosage of from 5 to 7 mg. per kg. employed in convulsive therapy in human beings. Six of the animals were killed by decapitation during the tonic stage of convulsions. One rabbit was permitted to survive the convulsions for 1 month before decapitation.

Discussion. Contrary to the findings of certain other investigators previously mentioned, we have observed that convulsant doses of metrazol given to dogs and rabbits may lead to organic pathologic changes. In the present study, the most important pathologic changes were observed in the central nervous system, especially the cerebral cortex (Figs. 1, 2, 3). More or less complete necrosis of the nervous parenchyma was observed in circumscribed areas; this was occasionally associated with slight glial reaction. In addition, there were diffuse degenerative changes of moderate degree in the

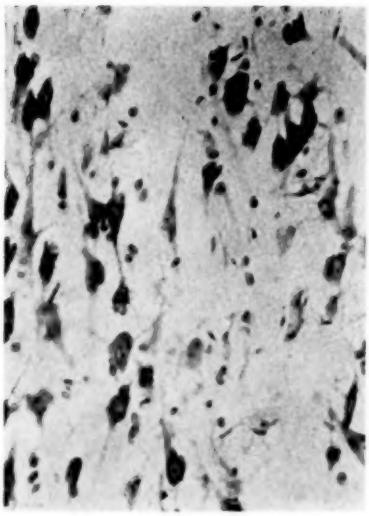

FIG. 1.—Area of recent ischemic necrosis in frontal cortex from Dog 3 which received 11 convulsant doses of metrazol. (Nissl × 300.)

FIG. 2.—Circumscribed area of recent ischemic necrosis in hippocampus, showing slightly more pronounced changes than Figure 1 from Dog 3 which received 11 convulsant doses of metrazol. (Nissl × 175.)

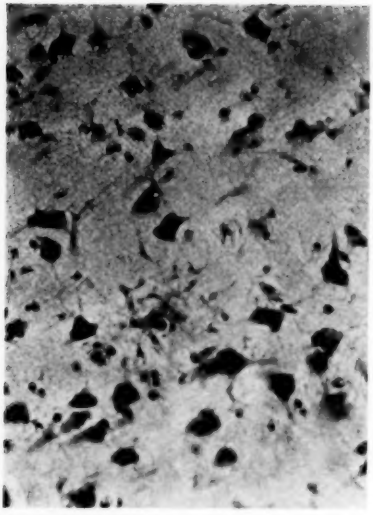

FIG. 3.—Neuronophagia in thalamus: proliferated glia cells surrounding a degenerating nerve cell, from Dog 3 which received 11 convulsant doses of metrazol (Nissl × 300.)

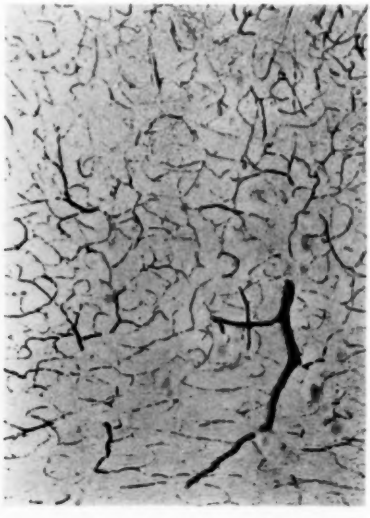

FIG 4.—Area of pronounced anemia in frontal cortex showing breaking-up of blood column in capillaries, from Dog 1 which received 14 convulsant doses of metrazol. (Benzidine stain [method of Doherty, Suh and Alexander[3]] × 125.)

TABLE I.—TABULATION OF RESULTS.

Animal		Convulsive

TABLE 1.—TABULATION OF RESULTS.

Protocol No.	Animal.		Experiment.			Dosage of metrazol.		Convulsive seizures.		Histologic findings at autopsy.			
	Sex.	Wt., kg.	Duration, days.	Injections.	Convulsant doses.	Range, mg. per kg.	Minimal convulsant dose, mg. per kg.	Number.	Duration.	Brain.	Other organs. Lungs.	Spleen.	Kidneys.
1 (Dog 1)	M	28.5	45	18	14	10–25	10	14	15 sec.-3 min.	Degenerative nerve cell changes in cortex: vacuolation, paleness, indistinct nuclear boundaries and very small nucleoli. In some areas of lower cortex: neuronophagia, "shadow" nerve cells, rod cells and slight proliferation of protoplasmic astrocytes. Some hyalinization in precapillary walls. (See also results of benzidine method in Discussion.)	Congestion; hemorrhages; atelectasis	Congestion	Congestion; tubular degeneration
2 (Dog 2)	F	9.0	45	17	16	15–30	15	16	30 sec.-3 min.	Changes similar in type and degree to those described in Proctocol 1.	Congestion; hemorrhages (massive); atelectasis	Congestion; hemosiderosis	Congestion; tubular degeneration.
3 (Dog 3)	F	12.8	51	19	11	12–35	25	11	30 sec.-7 min.	Pathologic changes most pronounced in series. In areas of cortex, hippocampus and thalamus: ischemic and "severe" nerve cell changes, pyknosis and ameboid glia cells. Generalized degenerative changes in gray matter: dislocation of cells, disappearance of Nissl bodies, vacuolation of cytoplasm, large pale nuclei with darkly stained chromatin particles, swollen and tortuous processes. Glial proliferation as in Protocol 1. Slight hyperemia. Endothelial proliferation in some cortical capillaries. Slight hyalinization of walls of a few cortical and meningeal arterioles and precapillaries.	Congestion; hemorrhages	Congestion	Congestion; tubular degeneration.
4 (Rabbit 1)	M	4.0	49	17	8	10–20	10	8	1 min.-1½ min.	Focal changes less numerous and less conspicuous than in dogs. Scattered nerve cell changes: tigrolysis, scleroses with tortuous dendrites, paleness, vacuolation, formation of intracellular rings (Nissl's "severe" change). Increased numbers of satellites, formation of "gliarasen." Some vascular dilatation.	Congestion (slight); atelectasis	Congestion (slight)	Congestion (slight); tubular degeneration.
5 (Rabbit 2)	M	2.62	45	15	11	10–30	15	11	45 sec.-3 min.	In some areas of lower cortex: paleness and disappearance of nerve cells, neuronophagia. Proliferation of protoplasmic astrocytes.	Congestion; hemorrhages; atelectasis	Congestion	Congestion; tubular degeneration.
6 (Rabbit 3)	M	2.50	45	14	11	10–30	15	11	25 sec.-2 min.	Pathologic changes less marked. Scattered nerve cell changes: paleness and vacuolation of cytoplasm, slightly increased prominence of dendrites. Glial proliferation as in Protocol 5.	Congestion; hemorrhages; atelectasis	Congestion	Congestion; tubular degeneration.
7 (Rabbit 4)	M	3.64	65* 29†	23	20	10–20	15	20	1 min.-2 min.	Pathologic changes more severe than in other rabbits. In areas of lower cortex: paleness and vacuolation of nerve cells, neuronophagia, even "severe" changes in a few cells. Glial proliferation as in Protocol 1. Moderate nerve cell changes throughout cortex. Slight hyalinization in vessel walls as in Protocol 1.	Congestion; hemorrhages; atelectasis	Congestion; hemosiderosis (slight)	Congestion; tubular degeneration.

* Injection period. † Recovery period.

nerve cells. Other changes observed consisted of slight tubular degeneration in the kidneys in all animals, scattered hemorrhages in the lungs, increased amounts of hemosiderin in the spleen, possibly increased amounts of glycogen in the liver and generalized visceral congestion.

In consideration of the nature of the above-mentioned lesions, one important factor in the pathogenesis appears to be a disturbance in the circulation. Further evidence confirming this view was obtained from the study of the brain of 1 dog (Dog 1) by means of the benzidine method.* The animal was killed at the height of the tonic stage of convulsions. Study of the brain revealed a breaking-up of the closed capillary network into irregular fragments (Fig. 4). The column of blood within the vessels was frequently interrupted. There was a generalized anemia which was more pronounced in some areas than in others. In a few scattered places there was an increased amount of blood. These results are in agreement with the previous more comprehensive study conducted by Dreszer and Scholz,[4] who employed the benzidine method in cats: an angiospastic-ischemic process is operative at the beginning of the convulsions. We are not able to state definitely whether this capillary spasm *per se* or a consequent stasis provokes the destruction of nerve cells. Comparative analyses of sections prepared by the benzidine and Nissl techniques may prove helpful. In places there is histologic evidence of hyperemia but not of stasis. We feel certain, therefore, that spasm of the capillaries is the principal circulatory disturbance involved and that this may be mainly responsible for the more or less circumscribed necrobiosis of the parenchyma. Leibel and Hall[14] have determined by direct measurement that during metrazol shock there is a decrease in cerebral blood flow, both arterial and venous, which is sufficient to produce a prolonged and severe cerebral anemia.†

In addition to the vascular disturbance another factor, anoxemia, appears to be operative in the pathogenesis of the lesions observed in the nerve cells. That metrazol convulsions produce considerable anoxemia is shown by the findings of Himwich and his co-workers,[11] who studied the blood changes during metrazol shock in human cases. During various stages of the convulsions the oxygen saturation of the blood fell to 42%. Even in the first moments after the convulsions ceased, the oxygen tension of the arterial blood did not reach the normal value. It is probable that the pathologic changes observed in the brain of the animals may, at least in part, be due to anoxemia. We may suppose that severe changes in the ganglion cells may arise at once incident to convulsions of 7 minutes' duration, as in Dog 3, and that convulsions of shorter duration, especially if

* The modification of Doherty, Suh and Alexander[3] was employed.

† Metrazol exerts a profound influence upon the circulation (Hildebrandt [review],[10] McDonald and Cobb,[15] Haury and Gruber[8]).

frequently repeated, may lead to less severe changes which are perhaps partly reversible.

Recent work by Wortis[24] shows that metrazol has no effect on the respiration of brain tissue *in vitro*. His results suggest that the harmful effect which metrazol has been shown to exert on the tissues of the central nervous system, as determined in the present work, is probably not due to a direct toxic action.

In summary, we believe that the evidence permits the assumption that interference with the blood flow to the brain and the anoxemia produced by the convulsions are the essential factors in causing the cerebral changes.*

The tissues of the rabbit which was allowed to survive 4 weeks after the last injection of metrazol did not exhibit any essential histologic differences from those of the other animals. There is no evidence of the formation of glia fibers nor of the production of scars. As yet, therefore, we are not able to say what becomes of foci of cellular destruction in the later stages. This rabbit, as well as Dogs 1 and 3, exhibited a very slight hyaline degeneration in the small cortical and pial vessels (arterioles and precapillaries). It may be assumed that repeated functional disturbances may favor the development of such changes in small vessels.

The true clinical significance of the brain changes observed in these experiments is not yet clear. The bulk of the nervous tissue appeared quite well preserved. The animals did not exhibit symptoms which could be referred to the brain changes.

The following clinical case shows correlative changes. A 26-year-old white male, who had received insulin-metrazol shock treatment for schizophrenia, made a fair recovery from his mental ailment but developed acute appendicitis which required operation. Postoperatively his condition was good and his temperature was normal. However, 2 months after the last injection of metrazol and 14 days after the last injection of insulin, the man suddenly died on the seventh postoperative day. At autopsy, 14 hours postmortem, death was shown to be due to pulmonary embolism incident to venous femoral thrombosis. Alterations in the brain were similar to those seen in the animals. The histologic findings were paleness and degeneration of scattered nerve cells and disappearance of a few cells in the hippocampus and neuronophagia in several cells, slight glial reaction and small "gliarasen" in the temporal cortex. Similar but less marked changes were observed in the frontal cortex, thalamus and interbrain. In places there was a slightly increased number of nuclei in the capillaries.

A second case of schizophrenia, in which convulsive metrazol therapy had been instituted, showed pathologic changes in the brain very similar to those mentioned in the above case. A 42-year-old

* Recent literature relating to the effects of anoxemia on the brain may be found in the work of Dellaporta,[5] Kabat and Dennis[13] and Gamper and Stiefler.[7]

white male died 15 days after the third injection of metrazol. Death occurred as a result of pyemia secondary to a left perirenal abscess for which nephrectomy had been performed 14 days previously. The brain changes observed were different from those usually found in pyemic conditions. Therefore, we believe these changes to be due to the action of metrazol.

In many other conditions, such as hypertension and epilepsy, similar small areas of cellular destruction have been observed frequently in different regions of the brain of patients, who during life presented no symptoms referable to these lesions.

In contrast to these observations, other studies indicate that metrazol shock therapy is not always without harm. A recent report indicates that this therapy may produce memory defects, especially for "neural patterns" of recent origin (Ziskind and Somerfeld-Ziskind[25]). This fact should enjoin caution in its use. It is known that individual patients may be highly susceptible to the convulsive action of metrazol. Therefore, care should be exercised in the institution of such therapy. It is probable that severe and irreversible changes in the brain may result from the administration of the usual metrazol dose to such individuals. Recently another and more serious danger has been shown to accompany metrazol shock treatment. When metrazol shock therapy was first introduced, dislocations were found to be a frequent accompaniment of the convulsions. Fractures including those of the bodies of the thoracic vertebræ have been described recently.[18] It seems quite probable that these serious accompaniments of the treatment will necessitate modification of metrazol treatment or substitution of some other less drastic form of therapy. A report indicating that metrazol therapy should probably not be used in individuals with cardiac disease has been mentioned previously (Hayman and Brody[9]). The employment of convulsive therapy in these patients may result in sudden death. Other toxic effects involve the hemopoietic system. Fatal aplastic anemia has been reported to follow metrazol shock therapy.[5]

Certain pathologic findings in organs other than the brain deserve comment. The hemorrhages in the lungs appear to be secondary to the convulsions. The presence of increased amounts of hemosiderin in the spleen suggests that hemorrhages may have occurred in previous convulsive attacks. In some cases of death in human beings during epileptic convulsions pulmonary hemorrhages have been observed by Neubürger[17b] and others. The slight degree of nephrosis is probably transient; the pathogenesis of this nephrosis is open to question.

Summary and Conclusions. Repeated administration of convulsant doses of metrazol to dogs and rabbits may lead to organic pathologic changes. The most important lesions were noted in the central nervous system, especially in the cerebral cortex. More or less complete necrosis of the nervous parenchyma was observed in

small circumscribed areas. This was occasionally associated with slight glial reaction and with diffuse degenerative change of moderate degree in the nerve cells. Other changes observed consisted of a mild degree of tubular degeneration in the kidneys, scattered hemorrhages in the lungs, increased amounts of hemosiderin in the spleen and generalized visceral congestion.

The possible etiology of the pathologic changes in the brain is discussed. Vascular spasm causing insufficient blood supply and anoxemia resulting from the convulsions are regarded as fundamental in the pathogenesis of the lesions produced by convulsant doses of metrazol.

Since this paper was submitted for publication, two articles dealing with brain changes following metrazol have come to our attention: **Hassin, G. B.**: Arch. Neurol. and Psychiat., **42**, 679, 1939; **Liebert, E.**, and **Weil, A.**: Ibid. p. 690.

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THE PRESSOR ACTION OF BENZEDRINE AND PAREDRINE.*

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Benzedrine and the closely related phenylpropylamines first attracted attention because of their ability to produce an increase in the blood pressure of experimental animals and in man.[2,7,17,22]

* This research was aided by a grant from Smith, Kline & French Laboratories.

Since the clinical report of Prinzmetal and Bloomberg in 1935[23] on the value of benzedrine in the treatment of narcolepsy more interest has been directed to the cerebral effects of this compound, and there has been a tendency to attribute all of its actions to central nervous system stimulation. The recent work of Myerson and his associates[20] also suggested that, in contrast to epinephrine, the blood pressure raising effect of benzedrine is not due primarily to peripherally mediated vasoconstriction. Paredrine,[1] a drug closely related to benzedrine, has been introduced recently; studies on the mechanism of its pressor action are few in number.

In the present work an attempt has been made to establish the site of action of these drugs. Two types of studies have been made:

1. Studies in man designed to test the effects of benzedrine and paredrine in patients in whom a marked fall in blood pressure occurred as a result of vasomotor paralysis due to spinal anesthesia. In most of these studies paredrine was the drug used, since the cerebral stimulating effect of benzedrine was undesirable under such circumstances.

2. Experiments based on the sensitivity of the central vasomotor centers to changes in the blood carbon dioxide concentration. Dale and Evans[10] showed that the activity of the bulbar vasomotor centers depends on the concentration of carbon dioxide in the blood, higher concentrations increasing the activity of these centers, while lower concentrations decrease their activity. On the other hand, changes in arterial blood carbon dioxide concentration do not affect vasoconstriction caused by the direct action of a drug on the smooth muscle of the arterial wall.[24a] It was therefore felt that the presence or absence of changes in blood pressure in response to changes in arterial blood carbon dioxide concentration in subjects in whom vasoconstriction had been induced by the action of benzedrine or paredrine would serve to localize the site of action of these drugs.

Methods. In the 10 studies of the action of the drugs in patients under spinal anesthesia 10 mg. of benzedrine or paredrine was given intramuscularly. In many instances the patients were pulseless. These studies were made in the operating room with the coöperation of the surgical service on patients in whom the systolic blood pressure had fallen to 60 mm. of mercury or lower following the induction of spinal anesthesia for various major operations.

In the 10 hyperventilation experiments the drugs were given orally in 30 or 40 mg. doses after the subject had rested in bed until his blood pressure became stable. Voluntary slow deep respiration was begun after the blood pressure reached a fairly constant high level, and was continued for 15 minutes. Alveolar air samples were taken by the Haldane technique at the beginning and end of hyperventilation and the carbon dioxide content was determined with the Haldane gas analysis apparatus. Blood pressure measurements were made at 2-minute intervals by the auscultatory method; the blood pressure level was noted for at least 10 minutes after the conclusion of hyperventilation. The pulse was counted frequently throughout the experiments.

Results. In patients in whom spinal anesthesia caused vasomotor paralysis and a marked fall in blood pressure, paredrine in every

instance raised the blood pressure to or above the pre-anesthesia level (Table 1). Similar results were noted in another group of patients following the injection of benzedrine.

TABLE 1.—EFFECT OF PAREDRINE AND BENZEDRINE ON BLOOD PRESSURE AFTER SPINAL ANESTHESIA.

Case.	B.P. before anesthesia.	B.P. before paredrine.	B.P. after paredrine.
1	120/75	30/0	150/80
2	150/80	40/20	180/120
3	130/80	60/40	160/60
4	150/110	50/0	160/80
5	100/60	0	90/60
6	130/80	0	120/80
7	100/60	0	100/60
8	120/60	50/20	160/70
		B.P. before benzedrine.	B.P. after benzedrine.
9	110/70	50/30	120/60
10	100/60	40/0	100/60

No significant fall in blood pressure occurred during or after voluntary hyperventilation (Table 2). In all instances the alveolar carbon dioxide content, and hence the arterial carbon dioxide content, was much diminished; mild tetany as manifested by numbness of the face and hands and the appearance of Chvostek's and Trousseau's signs occurred in 5 of the 10 subjects. The rises in blood pressure which occurred during the course of hyperventilation in several of the subjects were apparently due to the drug action since the elevation persisted after the end of forced breathing.

Discussion. Increases in systolic and diastolic blood pressure of the degree observed in the experiments here reported (Table 1) are similar to those which occur in essential hypertension and are due to arteriolar constriction. There is no basis for the statement frequently made that increases in cardiac output, blood volume, or blood viscosity elevate the blood pressure significantly, for normal values for blood pressure obtain in patients with increased cardiac output due to anemia and pregnancy,[12] in patients or animals with increased blood volume due to intravenous infusions,[4,26] and in patients with polycythemia vera[16] in which the blood viscosity may be increased to three or four times the normal value.

The results of the present study, which show that the blood pressure may be elevated markedly by the administration of benzedrine and paredrine in patients with peripheral vasomotor paralysis due to spinal anesthesia, indicate that these drugs act on the blood-vessel walls. Similarly, the observations that these drugs produce local vasoconstriction when applied to mucous membranes,[8] that they cause contraction of isolated strips of smooth muscle,[21] and that they retard the flow of blood through isolated animal tissues[14] point to the peripheral arterioles as the site of action of these drugs. Complete destruction of the brain and spinal cord[3] or paralysis of the sympathetic ganglia by means of nicotine or sparteine[27] does

not prevent a rise in blood pressure after administration of these drugs in animals. The failure of paredrine to produce blanching and cooling of the skin such as occurs after the administration of adrenalin[1] is not necessarily evidence against the presence of vasoconstriction elsewhere. Indeed there are available data which prove that the action of adrenalin on the skin is not an accurate measure of what happens in the rest of the body.[11]

TABLE 2.—EFFECT OF HYPERVENTILATION ON HYPERTENSION INDUCED BY BENZEDRINE AND PAREDRINE.

Patient.	Drug and dose.	Blood pressure.			Alveolar CO_2 (%).		Remarks.
		Basal.	Start of hyperventilation.	End of hyperventilation.	Start of hyperventilation.	End of hyperventilation.	
A. I. . .	30 mg. Benzedrine	120/78	154/98	156/96	5.17	2.73	Tetany
M. J. K.	30 mg. Benzedrine	104/60	136/84	138/84	5.03	2.60	Tetany
G. W. .	30 mg. Benzedrine	110/68	146/84	146/90	5.74	3.47	
J. W.	30 mg. Benzedrine	108/60	140/78	148/88	5.10	2.87	Tetany
G. G.. .	30 mg. Benzedrine	98/68	146/78	150/80	5.12	3.05	
E. C. .	30 mg. Benzedrine	92/72	132/80	128/86	5.23	2.12	Tetany
W. P. .	40 mg. Paredrine	125/60	160/78	168/82	5.14	3.28	
G. H. .	40 mg. Paredrine	118/68	144/78	154/86	5.36	3.68	
W. B. .	40 mg. Paredrine	106/74	156/80	146/86	5.00	3.53	
M. D. A.	40 mg. Paredrine	110/74	154/88	176/90	5.31	2.53	Tetany

The experiments in which hyperventilation was employed contribute suggestive but not conclusive data as to the site of action of the drug. Dale and Evans[10] and Raab[24b] have shown in animals that the activity of the bulbar vasomotor centers varies as the concentration of carbon dioxide in the blood perfusing these centers. These observations have been utilized clinically by Raab[24a] to differentiate centrally from peripherally affected vasoconstriction. He showed that lowering the arterial blood carbon dioxide concentration by means of hyperventilation lowered the blood pressure markedly in patients with essential hypertension but not in patients

with nephritic hypertension or hypertension due to the injection of adrenalin, a drug which acts on the peripheral arteries. The converse has also been observed clinically; inhalation of 10 % carbon dioxide raises the blood pressure more markedly in patients with essential hypertension than in normal subjects.[15] Similarly, holding the breath, which has been shown to cause a rapid increase in alveolar carbon dioxide concentration,[9] raises the blood pressure of patients with essential hypertension to a greater degree than that of normal subjects.[6] Since, according to Raab,[24a] hyperventilation lowers the blood pressure in hypertension when it is due to centrally mediated vasoconstriction but not when it is due to peripherally mediated vasoconstriction, hyperventilation was considered an additional means of localizing the site of action of benzedrine and paredrine. The fact that hyperventilation to the point of tetany failed to lower the blood pressure after it had been elevated by the administration of these drugs, is additional evidence pointing toward its peripheral action.

A number of foreign investigators have contended that paredrinol (Veritol), a methyl derivative of paredrine, raises the blood pressure by constricting the veins,[13,25,27] thereby squeezing blood into the arterial portion of the circulation. Whether or not such constriction of the veins occurs is at present being investigated in this laboratory, but even if appreciable venous constriction does occur, the increase in blood pressure cannot be explained on this mechanism since temporary or persistent[4,16] increases in blood volume do not have this effect.

The absence of generalized evidences of sympathetic activity, such as increase in metabolism, rise in blood sugar, increase in cardiac output, constant rise in pulse rate after the administration of benzedrine or paredrine,[5,18,19] is additional evidence against occurrence of central sympathetic stimulation. Similarly, the differences in the duration and time of onset and offset of cerebral stimulation and of blood pressure rise after the administration of benzedrine point to different sites of action. Benzedrine may produce marked psychic effects in the absence of measurable cardiovascular changes, while paredrine causes marked increase in blood pressure without cerebral stimulation.

Summary and Conclusions. 1. The marked pressor action of benzedrine and paredrine is due to arteriolar vasoconstriction.

2. Benzedrine and paredrine exhibit their usual pressor effects in patients in whom the vasomotor nerves of most of the body have been paralyzed by means of intraspinal nupercaine or novocaine.

3. Hyperventilation with its resultant marked decrease in arterial blood carbon dioxide concentration does not inhibit the pressor action of these drugs.

4. These observations are in accord with other evidence indicating that the smooth muscle of the arterioles is the site of the pressor action of these drugs.

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THE EFFECT OF SULFAPYRIDINE ALONE AND WITH SERUM ON PNEUMOCOCCIC PNEUMONIA AND ON PNEUMOCOCCUS-INFECTED MARROW CULTURES.*

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A considerable body of evidence has accumulated to indicate that *in vitro* the bacteriostatic and bactericidal action of sulfapyridine against the pneumococci[15a,21,24,28,37a] is superior to that of sulfanilamide. It has, moreover, been shown that sulfapyridine is capable of prevent-

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ing or curing pneumococcal infections in a variety of animals, and that this action is superior to that of sulfanilamide.[10,18,20,21,24,27,37a,33] This knowledge has been employed in the treatment of human pneumococcal infections, and reports have appeared showing the value of sulfapyridine in the treatment of the pneumonias and of other infections caused by the pneumococcus.[1–4,11–14,16,17,26,29,32,34–36,38] A number of workers have reported that specific serum increases the antistreptococcal and antipneumococcal activity of sulfanilamide, both *in vitro* and in experimental infections.[5,7,19,25,30] There have been several reports suggesting that specific immune bodies increase the antipneumococcal activity of sulfapyridine.[15a,28]

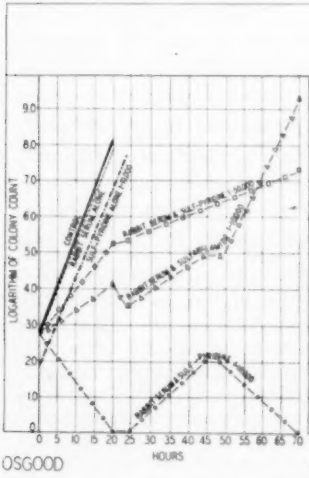
An evaluation of serochemotherapeutic and chemotherapeutic methods, by clinical means alone, would require considerable numbers of cases and years of observation. Though the clinic is the ultimate arbiter, the problem may be simplified and its elements may be separately analyzed and evaluated by laboratory approach. The technique of vaccine vial culture of human marrow permits controlled quantitative studies of the interaction of living cells, bacteria and therapeutic agents. This has already been demonstrated for sulfanilamide, with both streptococci and pneumococci.[30,31a,b] The present work represents an extension of original observations to include sulfapyridine and attempts to evaluate the importance of adding specific serum.

Method. The method of vaccine vial culture of human marrow has already been described.[31a] Human marrow serum medium at a buffered pH was infected with organisms. Equal portions were introduced into vials. The serum and sulfapyridine was then introduced through the stopper. Portions for examination were aspirated from time to time. The organisms used in the present investigations were strains recently isolated from pneumococcic pneumonia patients. The blood of patients was obtained by sterile technique, allowed to clot, and the serum centrifuged sterilely and refrigerated.

Results. A. Direct Effect in Marrow Cultures. (*a*) *Effective Dosage; Comparison With Sulfanilamide.* The control observations evidenced the same characteristics already described for the growth of pneumococci in marrow cultures.[30,31b] The usual growth of the pneumococcus under the conditions studied is demonstrated in Exp. 221 (Chart 1), performed on a freshly isolated virulent Type VII pneumococcus. Though as small an inoculum of this strain as 68 organisms per cc. was not sterilized by sulfapyridine alone (Exp. 220), a larger inoculum, 470 organisms per cc., was completely sterilized in 70 hours by sulfapyridine 10 mg. per 100 cc. plus 5 units of antiserum per cc. Sulfanilamide, 1 to 10,000, was practically without effect. Our general experience was that for the same size inoculum, concentrations of sulfapyridine equi-molar with 1 to 10,000 or 1 to 20,000 sulfanilamide were effective and more so than sulfanilamide or than the lower concentrations of sulfapyridine.

Because these concentrations are significantly bacteriostatic in marrow cultures and because they can be reached in the blood of human subjects (5 and 10 mg. per 100 cc. of blood), they were employed in the subsequent experiments. The effective concentrations of sulfapyridine alone in marrow cultures corresponded well with the observations of other workers, but is somewhat higher than that previously described for sulfanilamide against the streptococcus. An apparently effective concentration may not control all pneumococci, since there may be considerable variation in susceptibility of different strains to sulfapyridine.

Acetyl sulfapyridine had neither bactericidal nor bacteriostatic effect.



HOURS	0 (COLONY COUNT - ORGANISMS/C.C.)	20	24	44	48	69½
CONTROL	470	121 MILLION				
RABBIT ANTIPNEUMOCOCCUS SERUM*	470	72 MILLION				
RABBIT ANTIPNEUMOCOCCUS SERUM* PLUS SULFANILAMIDE 1-10,000	470	13500	3400	85000	75000	ACID**
RABBIT ANTIPNEUMOCOCCUS SERUM* PLUS SULFAPYRIDINE 1-10,000	470	0	0	>100	100	0
RABBIT ANTIPNEUMOCOCCUS SERUM* PLUS SULFAPYRIDINE 1-50,000	470	180000	238000	21 MILLION		
HORSE ANTIPNEUMOCOCCUS SERUM*	470	108 MILLION				
HORSE ANTIPNEUMOCOCCUS SERUM* PLUS SULFANILAMIDE 1-10,000	470	14000	3500	56000	71000	
HORSE ANTIPNEUMOCOCCUS SERUM* PLUS SULFAPYRIDINE 1-10,000	470	145	40	>100	100	0
HORSE ANTIPNEUMOCOCCUS SERUM* PLUS SULFAPYRIDINE 1-50,000	470	1445	1000	16000	14000	

* 5 UNITS PER CC.
** EXPERIENCE HAS SHOWN THAT WHEN A CULTURE IS ACID, THE COUNT HAS REACHED 200,000,000 ORGANISMS.

CHART 1.—Effect of sulfapyridine and sulfanilamide on Pneumococcus VII in marrow culture. Influence of added serum.

(*b*) *Lag Phenomenon.* In most of the experiments there was, immediately after inoculation, multiplication of the organisms for a period of several hours before bacteriostasis occurred (Charts 2 and 3). This phenomenon has been observed with sulfonamide compounds by several workers, particular reference having been called to it by Whitby.[37b]

(*c*) *The Effect of Type Specific Antibody.* The effect of both horse and rabbit sera on the bacteriostasis in marrow cultures containing sulfapyridine was studied. There was an equal effect for equivalent titers of antibody. The majority of our later experiments were performed with rabbit serum. Serum alone, in concentrations of 5 to 60 units per cc., induced, within the marrow culture, the usual specific phenomena of capsule swelling, agglutination and stimulation of phagocytosis. Serum alone had less effect on the growth

curves of the pneumococci than had been observed with the organisms used in the sulfanilamide experiments.[30] There was frequently diminished growth during the first 4 to 6 hours in the vials containing only specific serum, possibly due to agglutination (Chart 2), but this was generally followed by a rapid increase of growth, closely paralleling that of the control cultures.

In the majority of instances, bactericidal and bacteriostatic activity was appreciably increased when both serum and drug were present (Charts 1, 3 and 4–*b*). Where either very large or very

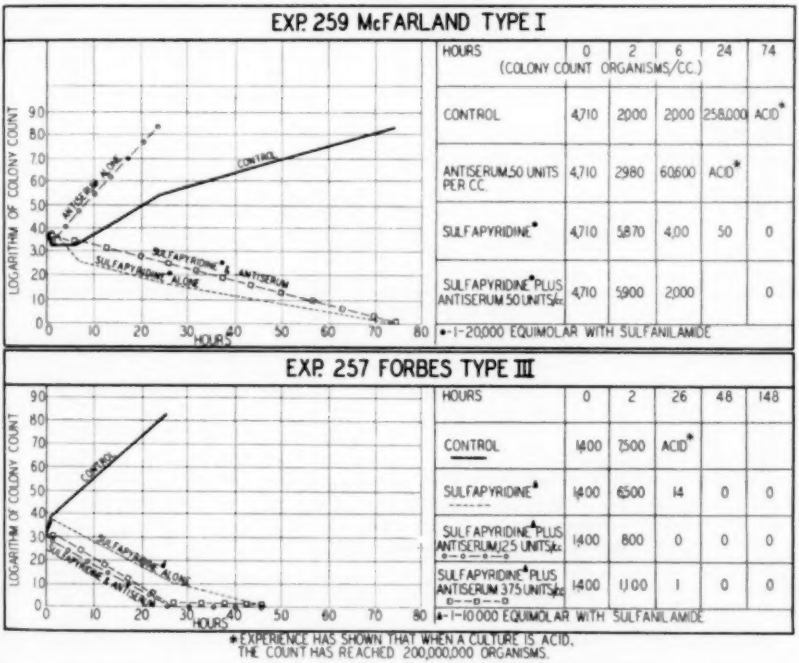

EXP. 259 McFARLAND TYPE I
LOGARITHM OF COLONY COUNT
HOURS
CONTROL
ANTISERUM ALONE
SULFAPYRIDINE ALONE
SULFAPYRIDINE PLUS ANTISERUM
HOURS (COLONY COUNT ORGANISMS/CC.) 0 2 6 24 74
CONTROL 4,710 2000 2000 258,000 ACID*
ANTISERUM 50 UNITS PER CC. 4,710 2980 60,600 ACID*
SULFAPYRIDINE* 4,710 5,870 4,00 50 0
SULFAPYRIDINE* PLUS ANTISERUM 50 UNITS/cc 4,710 5900 2,000 0
*-1-20,000 EQUIMOLAR WITH SULFANILAMIDE
EXP. 257 FORBES TYPE III
LOGARITHM OF COLONY COUNT
HOURS
CONTROL
SULFAPYRIDINE ALONE
SULFAPYRIDINE PLUS ANTISERUM
HOURS 0 2 26 48 148
CONTROL 1400 7500 ACID* 0 0
SULFAPYRIDINE* 1400 6500 14 0 0
SULFAPYRIDINE* PLUS ANTISERUM 12.5 UNITS/cc 1400 800 0 0 0
SULFAPYRIDINE* PLUS ANTISERUM 37.5 UNITS/cc 1400 1100 1 0 0
*-1-10,000 EQUIMOLAR WITH SULFANILAMIDE
*EXPERIENCE HAS SHOWN THAT WHEN A CULTURE IS ACID, THE COUNT HAS REACHED 200,000,000 ORGANISMS.

CHART 2.—(*a*) and (*b*). Pneumococcus I and Pneumococcus III in marrow cultures. Failure of serum to increase bacteriostatis.

small inoculations were used, however, the effect was less evident because, with the smaller inoculations, sulfapyridine alone was effective and with the very large inoculations neither the drug alone nor in combination with serum was capable of controlling the infection. Occasionally, however (Chart 5–*b*), an inoculum as large as 30,000 organisms per cc. of pneumococcus III was completely sterilized in 96 hours by sulfapyridine plus serum although it was not sterilized by sulfapyridine alone. Of interest in Exp. 252 (Chart 3) was the demonstration that, in the vial containing sulfapyridine plus serum, there were still 15 viable organisms per cc. at 54 hours.

That small numbers of organisms may remain alive for a long time in the presence of both sulfapyridine and serum, is of considerable importance in determining therapy. In several experiments, increasing antibody concentrations with the same concentrations of sulfapyridine, gave evidence of increasing bacteriostatic effects (Chart 3–*a*). This was not in proportion to the increase of antibody. Rarely the presence of antiserum inhibited the bacteriostatic activity of the sulfapyridine.

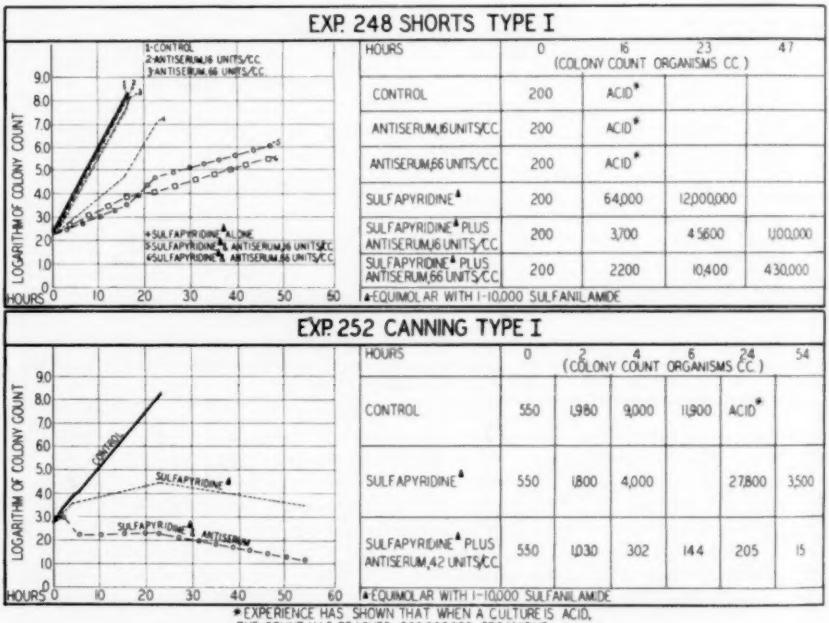

EXP. 248 SHORTS TYPE I
1-CONTROL
2-ANTISERUM,16 UNITS/C.C.
3-ANTISERUM,66 UNITS/C.C.
4-SULFAPYRIDINE▲ ALONE
5-SULFAPYRIDINE▲ + ANTISERUM,16 UNITS/C.C.
6-SULFAPYRIDINE▲ + ANTISERUM,66 UNITS/C.C.
LOGARITHM OF COLONY COUNT
HOURS
HOURS | 0 | 16 | 23 | 47
(COLONY COUNT ORGANISMS CC.)
CONTROL | 200 | ACID* | |
ANTISERUM,16 UNITS/C.C. | 200 | ACID* | |
ANTISERUM,66 UNITS/C.C. | 200 | ACID* | |
SULFAPYRIDINE▲ | 200 | 64,000 | 12,000,000 |
SULFAPYRIDINE▲ PLUS ANTISERUM,16 UNITS/C.C. | 200 | 3,700 | 45,600 | 1,00,000
SULFAPYRIDINE▲ PLUS ANTISERUM,66 UNITS/C.C. | 200 | 2200 | 10,400 | 430,000
▲EQUIMOLAR WITH 1-10,000 SULFANILAMIDE
EXP. 252 CANNING TYPE I
CONTROL
SULFAPYRIDINE▲
SULFAPYRIDINE▲ + ANTISERUM
LOGARITHM OF COLONY COUNT
HOURS
HOURS | 0 | 2 | 4 | 6 | 24 | 54
(COLONY COUNT ORGANISMS CC.)
CONTROL | 550 | 1,980 | 9,000 | 11,900 | ACID* |
SULFAPYRIDINE▲ | 550 | 1,800 | 4,000 | | 27,800 | 3,500
SULFAPYRIDINE▲ PLUS ANTISERUM,42 UNITS/C.C. | 550 | 1,030 | 302 | 144 | 205 | 15
▲EQUIMOLAR WITH 1-10,000 SULFANILAMIDE
*EXPERIENCE HAS SHOWN THAT WHEN A CULTURE IS ACID, THE COUNT HAS REACHED 200,000,000 ORGANISMS.

CHART 3.—(*a*) and (*b*). Two strains of Pneumococcus I showing increased susceptibility to sulfapyridine in the presence of serum.

(*d*) *The Effect of Variation in Virulence.* Experiments 221, 223, 227 and 228 (Chart 6) were performed with the same strain of a Type VII organism. Approximately 4 weeks elapsed between the first and last observations, during which time the organism had been subcultured on blood agar slants. A progressive increase in the organisms' susceptibility took place, and in later experiments (228), large inoculations were completely sterilized by concentrations of sulfapyridine alone which had failed in the first experiments. Moreover, in the initial experiment (Chart 1) antiserum in addition had been necessary to sterilize. Suspensions containing 10,000 organisms in the stage used in Exp. 228 were unable to kill mice. For this organism at least, the increased vulnerability to the drug

was associated with a considerable decrease in virulence for mice. These observations indicate that firm conclusions must not be based upon comparisons involved in a single experiment, and, when several experiments are considered, the inferences can be only tentative.

(*e*) *Strain Variations.* The existence of variations in susceptibility to sulfapyridine, already indicated, suggests the possibility that there might also be variations among different strains of the same type of organism in the same stage of isolation. The following comparative observations were not made simultaneously and, while

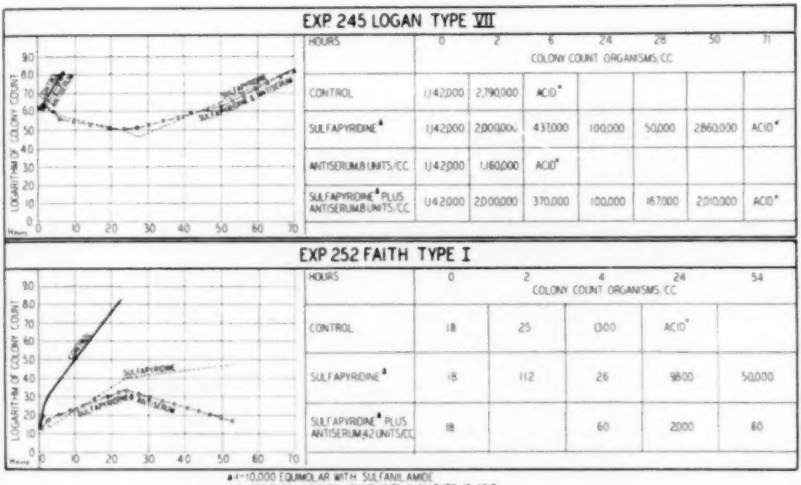


CHART 4.—(*a*) Large inoculum of Pneumococcus VII showing temporary bacteriostasis and later growth. (*b*) Failure of sulfapyridine to sterilize small inoculum despite added Pneumococcus I serum.

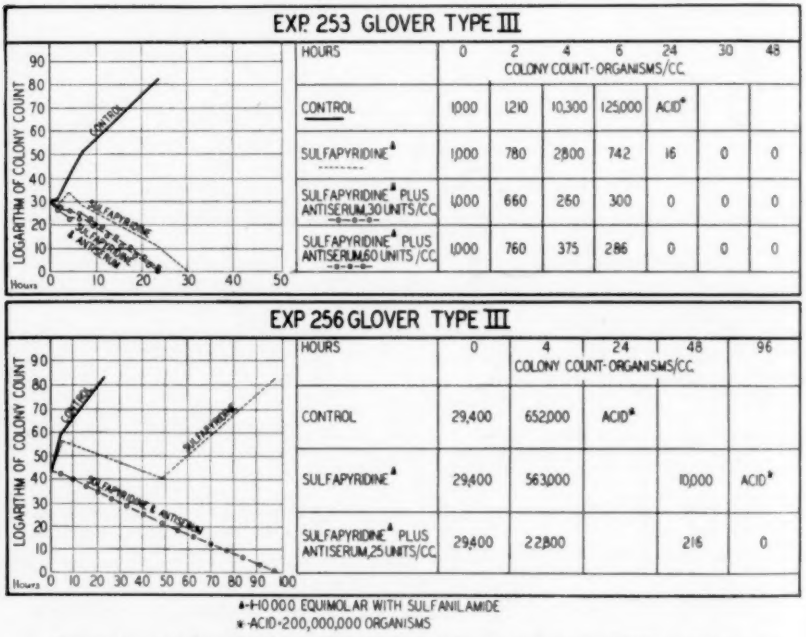
conclusions based upon them must be guarded, we may, nevertheless, tentatively accept that there are variations in susceptibility of different strains of pneumococci to sulfapyridine. The recent observations of MacLean, Rogers and Fleming[28] support this view.

A Type I strain (Faith) could not be sterilized in an inoculum of only 18 organisms per cc. by sulfapyridine and serum in 54 hours (Chart 4). Another Type I strain (Garret), in an inoculum of 700 organisms per cc., was completely sterilized by sulfapyridine alone in less than 74 hours.

(*f*) *Effect of Size of Inoculum.* That the size of the original inoculum is an important factor in determining the outcome of an experiment, is indicated in Chart 5. One thousand organisms per cc. of a Type III strain (Glover) were sterilized by sulfapyridine alone in 30 hours, while 30,000 organisms of the same strain in a later experiment could be sterilized only by sulfapyridine and serum after

96 hours. This would tend to show that concentrations of sulfapyridine which are perfectly adequate for smaller inocula of some strains may be inadequate when larger inoculations of the same strain are used.

With extremely large inocula (which were made to assure a sufficient growth so that adequate morphologic observations could be made) it was interesting to note that temporary bacteriostasis might occur, only to be followed by ultimate growth of the marrow culture to acidity (Chart 4–*a*). These observations are in agreement with the quantitative studies of McIntosh and Whitby,[27]



EXP. 253 GLOVER TYPE III							
HOURS	0	2	4	6	24	30	48
	COLONY COUNT-ORGANISMS/CC.						
CONTROL	1000	1210	10,300	125,000	ACID*		
SULFAPYRIDINE†	1,000	780	2800	742	16	0	0
SULFAPYRIDINE† PLUS ANTISERUM 30 UNITS/CC	1,000	660	260	300	0	0	0
SULFAPYRIDINE† PLUS ANTISERUM 60 UNITS/CC	1,000	760	375	286	0	0	0

EXP 256 GLOVER TYPE III					
HOURS	0	4	24	48	96
	COLONY COUNT-ORGANISMS/CC.				
CONTROL	29,400	652,000	ACID*		
SULFAPYRIDINE†	29,400	563,000		10,000	ACID*
SULFAPYRIDINE† PLUS ANTISERUM 25 UNITS/CC.	29,400	22,800		216	0

†=10,000 EQUIMOLAR WITH SULFANILAMIDE
*=ACID-200,000,000 ORGANISMS

CHART 5.—(*a*) Successful sterilization of moderate inoculum (1000 organisms per cc.). (*b*) Very large inoculum (29,400 organisms) successfully sterilized only after serum was added.

who demonstrated the necessity of increasing concentrations of sulfapyridine for the sterilization of larger inocula of the same organism. The relationship in our experiments, however, did not seem to have the direct proportions of a quantitative chemical reaction.

B. EFFECT OF VARIATIONS IN THE MEDIUM. The influence of variations in the culture medium on the bacteriostatic and bactericidal action of sulfapyridine has attracted considerable attention. This, like the lag phenomenon, bears upon the highly important problem of the mechanism of action of the drug. We failed to

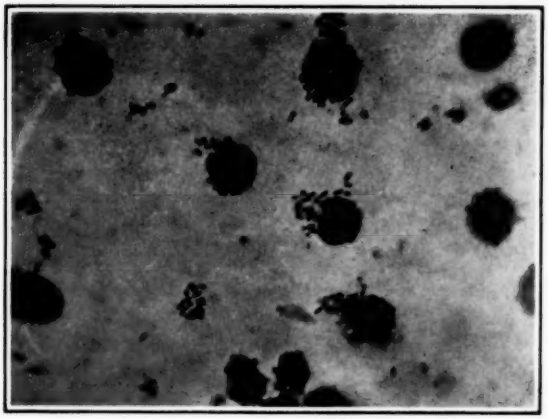


FIG. 1*a*.—Exp. 258. Pneumococcus VII. Control at 24 hours. (Wright stain smear from marrow cultures, × 1200.) (Courtesy of Spencer Lens Company.)

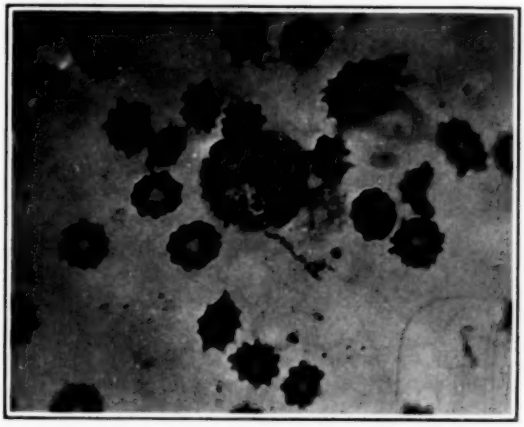


FIG. 1*b*.—Exp. 258. Pneumococcus VII. Sulfapyridine, 1 to 20,000 at 24 hours. Lysis, chain formation phagocytosis

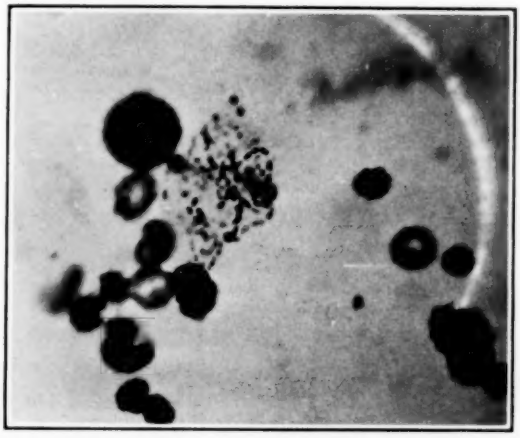


FIG. 1*c*.—Exp. 248. Pneumococcus I. Sulfapyridine, 1 to 10,000 plus 66 units serum per cc., 16 hours. Distortion, lysis and chain formation.

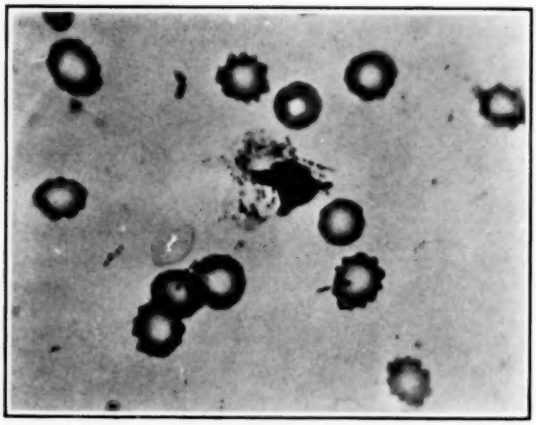


FIG. 1*d*.—Exp. 258. Pneumococcus VII. Sulfapyridine, 1 to 20,000, plus antiserum, 50 units per cc., 24 hours. Lysis distortion and chain formation.

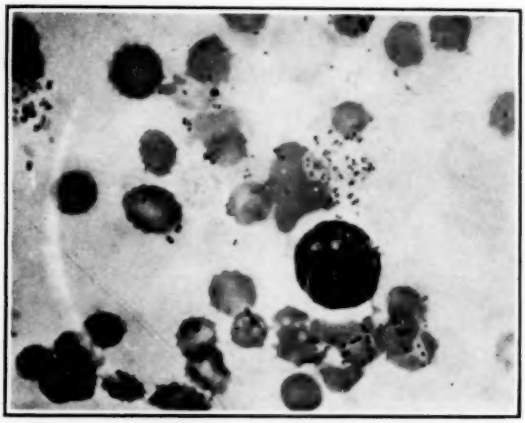


FIG. 1*e*.—Exp. 258. Pneumococcus VII. Antiserum, 50 units per cc., 24 hours. Agglutination and capsule swelling.

observe inhibition of bacteriostasis due to added peptone, as reported by Lockwood. Our experience is in agreement with the observations of McIntosh and Whitby.[27] Phagocytosis was apparently not essential in determining the outcome of therapy because sulfapyridine accomplished complete sterilization of pneumococci inoculated into Hartley broth.

C. Morphologic Changes Under Influence of Drug and Serum. 1. *In Marrow Culture.* Marked morphologic changes in streptococci exposed to sulfanilamide have been observed.[7,23] A

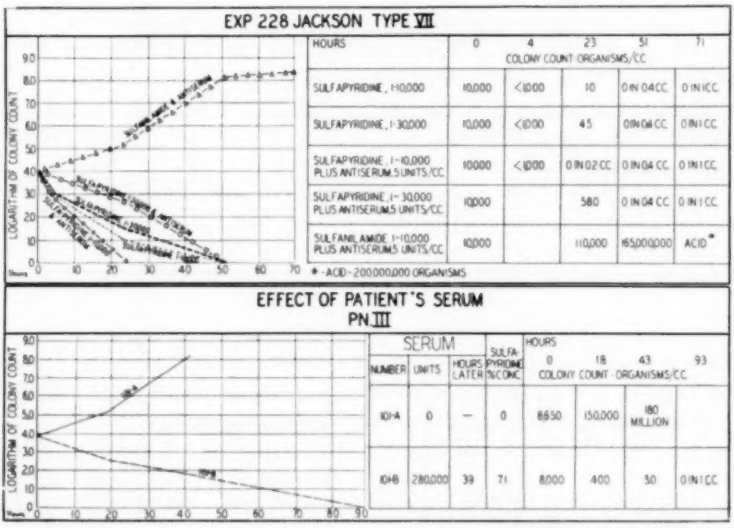


Chart 6.—(*a*) Increased vulnerability of Pneumococcus VII, same strain as in Exp. 221, after 1 month's cultivation in agar. (*b*) Exp. 220. Effect of patient's serum before and after treatment with serum and sulfapyridine.

report has appeared ascribing to sulfapyridine, morphologic alterations in the pneumococci obtained from treated patients.[36] Loss of capsular substance with a consequent loss of type specificity has been described. This has not, however, been confirmed by others so that doubt has been thrown upon the observations.[15b,21,28]

In our study of morphologic changes in marrow cultures, large inocula were made and Wright stain smears examined at various intervals (Fig. 1–*a*, *b*, *c*, *d* and *e*). In general, morphologic alterations were observed after some hours of exposure to the drug. These changes consisted of a tendency to chain formation, swelling and distortion of the organisms, and actual lysis. In the presence of specific antiserum capsule swelling occurred. Agglutination of organisms also occurred when antiserum was present, indicating that type specificity was retained. At the same time, the control cultures, with more bacteria than those containing sulfapyridine,

showed good morphology, indicating that the morphologic changes were not due either to exhaustion of the media or to the production of metabolites. Phagocytosis also was not responsible for morphologic changes because it was present in the control cultures to the same degree as in cultures containing the drug.

2. *In Patients.* In a number of instances, sputa from pneumonia patients treated with sulfapyridine were typed at intervals during the course of treatment. We were always able to obtain the type

PN. I INFECTED MARROW CULTURE

EFFECT OF PATIENT'S SERUM

PATIENT	SERUM UNITS	SERUM HOURS LATER	SULFAPYRIDINE % CONC.	HOURS 0	17	21	41	45½	92
				ORGANISMS/CC (COLONY COUNT)					
107-A	0	–	6.0	A) 10	2			148	100,000,000
				B) 17,700		<100,000,000			
142-A	0	–	6.7	A) 5	0			0	0
				B) 17,700		200,000,000	<100,000,000		
102-A	420,000	36	8.0	A) 10	0			0	0
				B) 17,700		80,000	<100,000,000		
143-A	156,000	36	8.3	A) 5	0			0	0
				B) 17,700		40,000	60,000,000		<100,000,000

OSGOOD

A-WASHINGTON PN.I CULTURE
B-VARGAS PN.I CULTURE

CHART 7.—Relative bactericidal activity of serums from treated patients.

from these sputa, either by direct examination (Neufeld), or by mouse inoculation. In 1 instance of Type VIII pneumonia, pneumococci continued to be typed directly from the sputum for 8 successive days of treatment with sulfapyridine. This patient recovered. In a fatal pneumococcal Type XVIII meningitis treated with sulfapyridine and both intravenous and intrathecal specific antiserum, capsules were seen on direct examination of the spinal fluid without addition of typing serum, for a period of 5 days;

throughout all of this time the spinal fluid concentration of sulfapyridine ranged between 5 and 9 mg. per 100 cc.

D. Relative Antibacterial Power of Blood Obtained From Patients. Colebrook, Buttle and O'Meara[9] have demonstrated that treatment with sulfanilamide confers increased bactericidal and bacteriostatic properties upon the blood and serum of humans. Fleming[15b] demonstrated this phenomenon with human blood obtained from patients receiving sulfapyridine, and showed that the increased bactericidal property was in the serum rather than in the cells. We studied the bactericidal property of 10 specimens of human serum obtained from patients of several types, in some cases before treatment, but in most instances after treatment with either serum, sulfapyridine, or the combination. These sets of sera were subjected to studies in marrow cultures at the same time, using the same organism and, whenever possible, the same size inoculum. Although we did not determine the relative bactericidal power of normal human sera in marrow cultures, Colebrook, Buttle and O'Meara[9] have already shown that, although human whole bloods from various individuals differ in their bactericidal action against the streptococcus, there was practically no variation when only serum was used.

The serum of patients treated with sulfapyridine was considerably less bacteriostatic than the serum from those patients treated with both serum and sulfapyridine. Serum from patients given therapeutic serum alone had practically no bacteriostatic activity. Serum from untreated patients exhibited no significant bacteriostatic or bactericidal activity. Experiment 220 (Chart 6) clearly demonstrates the difference in sera obtained from a Type III patient before treatment and after treatment with both serum and sulfapyridine. Chart 7 demonstrates the same phenomenon with different sizes of inocula. There was increased bactericidal activity in the presence of both serum and drug.*

E. Statistical Results of Therapy in Human Infections. During the past 4 months we have rotated in treatment patients with pneumococcic pneumonias, immediately after type identification, in order to evaluate the effect of serum, sulfapyridine, or the combination; 324 adults and 113 children were studied, as shown in the tables 1, 2, 3.

* Since this article went to press, Spring, Lowell and Finland (J. Clin. Invest., 19, 163, 1940) have reported observations on the effect of sulfapyridine on the growth curve of the pneumococcus *in vitro*. Their studies also revealed the presence of a lag phase before bacteriostasis due to sulfapyridine, diminishing bacteriostatic effect with increasing size of inoculum, and a failure of peptone to inhibit bacteriostasis due to sulfapyridine. In their pneumococcidal studies the addition of specific immune serum was also found to increase the bacteriostatic and bactericidal activity of sulfapyridine. They obtained results essentially similar to ours in studies of the pneumococcidal activity of bloods obtained from treated patients, concluding that the pneumococcidal activity was proportional to the sulfapyridine and antibody content of the blood specimens obtained.

The death rate in adults, for all types, with sulfapyridine alone, was significantly lower than that for serum alone. Curiously, the death rate with serum plus sulfapyridine was somewhat higher than that for the drug alone. This is at variance with the experimental results reported by others and with our own observations in marrow cultures. However, when the statistics are broken down, it becomes evident that the lowest death rate in early treated cases (1 to 4 days) was obtained with the combination of serum and sulfapyri-

TABLE 1.—NEW YORK UNIVERSITY COLLEGE OF MEDICINE (LITTAUER FUND), HARLEM HOSPITAL, JANUARY 1, 1939–MAY 1, 1939.

ADULTS.

Type.	Serum.			Sulfapyridine.			Serum and sulfapyridine.		
	Cases.	Deaths.	%.	Cases.	Deaths.	%.	Cases.	Deaths.	%.
I	25 4*	1 0*		24 7*	0 0*		20 2*	1 0*	
II	4 1*	0 0*		2 0*	1 0*		2 1*	0 0*	
III	11 2*	4 2*		12 1*	1 1*		10 2*	2 2*	
V	10 0*	1 0*		10 4*	3 3*		8 1*	1† 1*	
VII	18 4*	5 3*		18 1*	0 0*		18 1*	2 1*	
VIII	7 2*	1 0*		7 2*	1 1*		5 0*	1 0*	
Other types‡	36 4*	7 3*		52 1*	4§ 0*		26 1*	3 1*	
Total	111 17*	19 8*	17.3 47*	124 16*	10 5*	8.1 31*	89 8*	10 5*	11.2 62.5*
				R.D.E. = 2.1		R.D.E. = 0.7			
				R.D.E. = 1.2					

* Bacteremia.
† Bacteremia controlled, congestive failure.
‡ Including multiple infections.
§ Pneumonia controlled, gastro-intestinal malignancy (1 case).

dine. These findings are in agreement with the recent experimental observations of Kepl and Gunn[22] on rats. They found that serum plus sulfapyridine was the most efficient therapeutic agent when treatment was begun soon after infection, and that in animals treated late the addition of serum did not augment the therapeutic effects obtained with sulfapyridine.

Final conclusions regarding the effect of drug and antibody in the treatment of the pneumonias must await additional observations.

Comments and Discussion. Most of the work done on sulfanilamide and its derivatives has, for its goal, the establishment of a rational therapy. In this regard, we have reason to believe that the same principles which govern the therapy of streptococcal infections with sulfanilamide will also govern the therapy of pneumococcal infections with sulfapyridine. It has been well established that specific serum markedly improves the sulfanilamide therapy of

TABLE 2.—NEW YORK UNIVERSITY COLLEGE OF MEDICINE (LITTAUER FUND), HARLEM HOSPITAL, JANUARY 1, 1939–MAY 1, 1939.

CHILDREN.

	Serum.			Sulfapyridine.			Serum and sulfapyridine.		
	Cases.	Deaths.	%.	Cases.	Deaths.	%.	Cases.	Deaths.	%.
Total . .	**37** 4*	**1** 0*	**2 7** 0.0*	**50** 0*	**2** 0*	**4.0** 0.0*	**26** 1*	**1** 0*	**3.8** 0.0*
Age.									
Under 2 yrs.	17 3*	1 0*	5.9 0.0*	22 0*	2 0*	9.1 0.0*	13 1*	1 0*	7.7 0.0*
2 to 12 yrs.	20 1*	0 0*	0.0 0.0*	28 0*	0 0*	0.0 0.0*	13 0*	0 0*	0.0 0.0*
Pn. XIV .	11 3*	0 0*	0.0 0.0*	10 0*	0 0*	0.0 0.0*	11 1*	0 0*	0.0 0.0*
Lobar . .	32 4*	1 0*	3.1 0.0*	42 0*	0 0*	0.0 0.0*	25 1*	1 0*	4.0 0.0*
Broncho. .	5 0*	0 0*	0.0 0.0*	8 0*	2 0*	25.0 0.0*	1 0*	0 0*	0.0 0.0*

* Bacteremia.

experimental infections. Lowenthal[25] found that serum plus sulfanilamide cured 75% of mice infected with streptococci while sulfanilamide alone cured 11% and serum alone cured none. Branham and Rosenthal[5] similarly found that the recovery rate for serum plus sulfanilamide in experimental pneumococcus infections was greater than for either alone and could not be accounted for by a simple addition of effects. Chandler and Janeway[7] demonstrated *in vitro* an increased bacteriostasis of the streptococcus by sulfanilamide in the presence of immune serum. MacLean, Rogers and Fleming[28] have shown that a single dose of pneumococcal vaccine combined with the oral administration of sulfapyridine saved mice that would inevitably have died if treated by either method alone; this was ascribed to the synergistic action of vaccine and drug. Agranat, Dreosti and Ordman[1] demonstrated that the

recovery from pneumonia occurred more quickly with sulfapyridine in those natives who had previously been vaccinated with pneumococci. Plummer and Ensworth[32] reported a mortality rate in cases treated with sulfapyridine and serum lower than that for sulfapyridine alone. Our observations also indicate that serum plus sulfapyridine is a more effective therapeutic agent than either acting alone in the early cases when autogenous antibody cannot be expected to be present. Furthermore, Bukantz, Bullowa and de Gara[6] have recently suggested that serum plus sulfapyridine may be essential when the severity of a pneumococcal infection is so great that capsular polysaccharide is detected in the blood.

TABLE 3.—NEW YORK UNIVERSITY COLLEGE OF MEDICINE (LITTAUER FUND), HARLEM HOSPITAL, JANUARY 1, 1939–MAY 1, 1939.

ADULTS.

Age.	Serum.			Sulfapyridine.			Serum and sulfapyridine.		
	Cases.	Deaths.	%.	Cases.	Deaths.	%.	Cases.	Deaths.	%.
Under 40 yrs.	61 8*	8 3*	13.1 37.5*	72 9*	4 2*	5.6 22.2*	52 3*	3 1*	5.8 33.3*
				R.D.E. = 1.5					
40 years and over	49 9*	11 5*	22.5 55.7*	52 7*	6 3*	11.5 43.0*	37 5*	7 4*	19.0 80.0*
				R.D.E. = 1.5					
Day of disease: 1st to 4th day	57 9*	7 4*	12.3 44.5*	53 5*	5 2*	9.4 40.0*	39 2*	1 0*	2.6 0.0*
					R.D.E. = 1.94				
5th day and over + ?	53 8*	12 4*	22.7 50.0*	71 11*	5 3*	7.1 27.3*	50 6*	9 5*	18.0* 83.3
				R.D.E. = 2.4					

* Bacteremia.

There are many hazards in the attempt to make accurate interpretations of chemotherapeutic experiments. Chesney,[8] many years ago, demonstrated that, prior to the development of an active logarithmic phase of growth, there was a definite lag in the multiplication of organisms *in vitro*. It would certainly seem that an organism subjected to the action of a chemical agent while in a lag phase of growth, *in vitro*, might be inhibited in its growth to a greater extent than when in its logarithmic phase. Under such conditions, an unwarranted degree of activity might be attributed to the influence of the drug. Nevertheless, it appears as though few investigators of sulfapyridine have observed the precaution of inoculating organisms in the active logarithmic phase of growth. In McIntosh and Whitby's[27] experiments, a highly virulent organism in the active logarithmic phase of growth was always used. In their experiments non-immune mice inoculated with pneumococci inevitably developed bacteremia despite sulfapyridine therapy. Immune

mice, on the other hand, inoculated with the same number of organisms, failed to develop bacteremia. These differences in behavior occurred despite the fact that both groups of mice were ultimately cured.

Our own observations have indicated that pneumococci may remain viable for as long as 54 hours, despite the presence of sulfa-

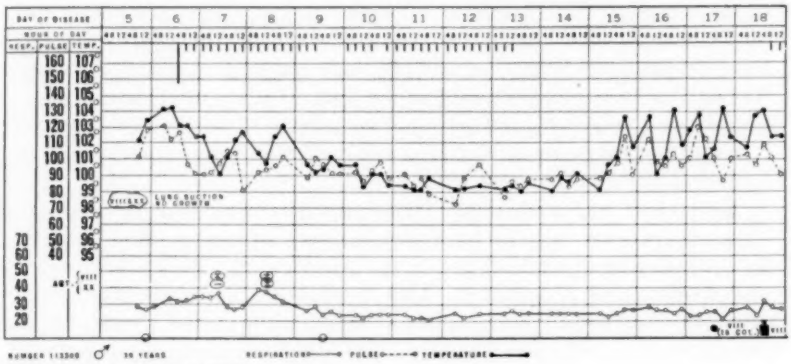


CHART 2 (*a*).

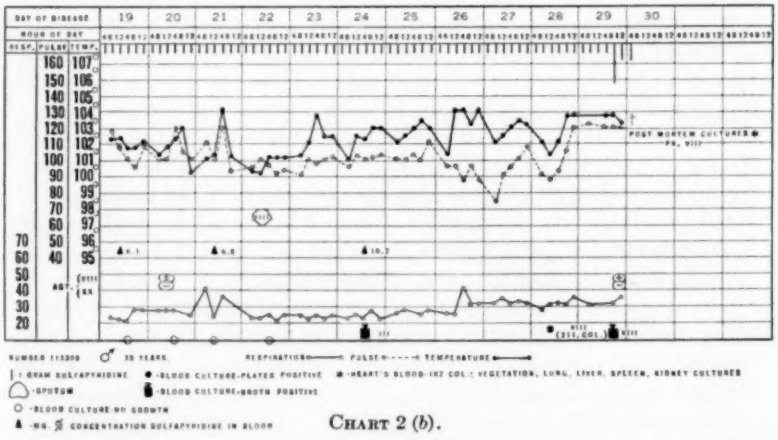


CHART 2 (*b*).

CHART 2.—(*a*) and (*b*). Fever charts of patient in whose sputum Pn. VIII and Pn. XX were found. Forty-eight hours after discontinuing sulfapyridine, the temperature rose. The blood became invaded with Pn. VIII; signs of aortic valvular disease developed. The condition was uncontrolled by subsequent administration of sulfapyridine. Agglutinins were present and the blood culture positive simultaneously. Postmortem: vegetative endocarditis.

pyridine and serum, and that large inocula may undergo temporary bacteriostasis with subsequent overgrowth. The significance of such observations to human therapy is well indicated by the following case whose fever chart is shown in Chart 2. A man of 39 was

treated with sulfapyridine on the 6th day of his illness, receiving the drug for the next 7 days. His temperature was below 100° F. on the 12th and 13th days, when the drug was stopped. On the 14th day the temperature reached 100° F.; on the 15th day the temperature reached 103.8° F., and continued until the 18th day, at which time a blood culture taken on the 17th day grew a pneumococcus VIII. Previous blood cultures had been sterile. In spite of the continuation of sulfapyridine therapy and the development of agglutinating antibodies, this patient died on the 29th day and autopsy confirmed the diagnosis of bacterial endocarditis. Whitby had earlier shown that sulfapyridine had no effect upon the quality, quantity or speed of production of specific antibodies, and felt that it would be unwise to sacrifice the methods of immune therapy which had already been proven valuable.

That there are additional difficulties in attempting to evaluate chemotherapeutic agents by animal experimentation, are indicated by the differing reports of such workers as Cooper, Gross and Lewis,[10] and Hilles and Schmidt.[20] The former workers reported little advantage of sulfapyridine over sulfanilamide in the treatment of pneumococcal infections in mice and pneumococcal meningitis in rats. However, the treatment dose employed by them was small, only 20 mg. of either drug at 3, 23, 47 and 72 hours after infection for the mice, and 20 mg. at 6, 13 and 23 hours after infection followed by 20 mg. once daily for 8 doses in the rats. By these methods of therapy, sulfapyridine proved to be of only slightly greater therapeutic action than sulfanilamide. Hilles and Schmidt, working with Type XXII infections in mice, found that with a dosage of 20 mg. orally at 2, 6, 12, 18 and 24 hours with an additional 24-hour, 20-mg. dose for 5 successive days, only 1 out of 20 mice survived for 30 days, though life was slightly prolonged in the remaining 19. Where therapy was changed to 20 mg. 2 hours after infection, then every 6 hours for 4 days, then at 12-hour intervals for 2 days, 14 of 20 mice lived for 30 days, and of the remaining 6, 4 died after discontinuance of therapy. Raiziss, Severac, Moetsch and Clemence,[33] using an even less adequate dosage than Cooper, Gross and Lewis, concluded also that sulfapyridine was not much more effective than sulfanilamide. Long, Bliss and Feinstone,[24] using a dosage intermediate between that of Cooper, Gross and Lewis, and Hilles and Schmidt, reported intermediate results in the therapy of pneumococcal infections in mice.

Some uniform standard dosage, as well as strains of uniform resistance and in the same phase of growth, should be employed in order that there may be accurate comparable results. This is of particular importance to the clinician who desires information in order to establish the most efficient form of therapy. For the present we must conclude that the optimal conditions and combinations in our therapy of the pneumococcal pneumonias have not been deter-

mined. Considerably more careful work must be done before the rôle of serum and of sulfapyridine can be formulated.

Summary and Conclusions. 1. In marrow cultures, the presence of sulfapyridine in concentrations of 5 mg. or more per 100 cc. may lead to ultimate sterility with inocula of 500 or less per cc. of Types I, III or VII pneumococci, whereas controls uniformly grow out to numbers of over 100 million colonies per cc.

2. The larger the initial inoculation, the less likely is sulfapyridine to lead to sterility.

3. Sulfapyridine alone is definitely more effective against pneumococci than equi-molar concentrations of sulfanilamide.

4. The presence of type specific antiserum almost always increases the effectiveness of sulfapyridine. With the same concentrations of sulfapyridine, increasing quantities of antibody are increasingly effective. Occasionally, for some undetermined reason, the presence of serum interferes with the bactericidal action of sulfapyridine *in vitro*.

5. Within the range of concentrations employed varying from 1 to 10,000 to 1 to 100,000, an increase in concentration of sulfapyridine results in an increase in effectiveness.

6. Different strains of pneumococci appear to vary in their susceptibility to the action of sulfanilamide and sulfapyridine. Freshly isolated virulent strains appear to be less susceptible than strains after they have been grown for some time in ordinary media.

7. The presence of sulfapyridine does not result in rapid death of all organisms present. Small numbers of living organisms may be present for several days in cultures which appear microscopically sterile.

8. Sulfapyridine, in the concentrations employed, has not yet appeared to damage directly the marrow cells nor to favor or inhibit phagocytosis.

9. Organisms exposed to the action of sulfapyridine undergo no loss of capsule or type specificity. They may, however, become distorted, develop long chains and stain irregularly.

10. Acetyl sulfapyridine is ineffective.

11. Serum from untreated pneumonia patients is not bactericidal. The serum from treated patients has bactericidal activity roughly proportional to sulfapyridine and serum content.

12. Sulfapyridine alone gave a lower death rate than either serum alone or serum plus sulfapyridine, in a series of 324 adult patients rotated for treatment. The lowest mortality rate was observed in cases treated early (1 to 4 days) with serum and sulfapyridine. The results in children were inconclusive and in infants did not show the trend in adults.

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A FATAL CASE OF HEMOLYTIC ANEMIA AND NEPHROTIC UREMIA FOLLOWING SULFAPYRIDINE ADMINISTRATION.

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Sulfapyridine ("Dagenan" or "M & B 693") recently released to the medical profession, has rapidly come into fore and has in many institutions almost entirely superseded serum therapy in the treatment of pneumonia. It is therefore pertinent at this stage of the problem to report all known cases wherein toxic or even fatal results were obtained with this drug. The present case is, we think, the first of an acute fatal hemolytic anemia and renal insufficiency as caused by sulfapyridine to be reported in the literature.

Case Report. H. L., a 79-year-old white man, was admitted to this hospital on March 17, 1939, with the chief complaint of abdominal distention and vomiting of 4 days' duration. On *physical examination* he appeared to be an acutely ill old man. Abdomen was markedly distended, but not

tender. Basal râles were heard in the left lung. (T. 100° F.; P., 100; R., 24; B. P. 164/100). *Blood examination* on admission showed a leukocyte count of 19,000 per cc. (84% neutrophils). *Blood chemistry:* glucose, 144.6 mg.; urea nitrogen, 24.4 mg.; non-protein nitrogen, 49.4 mg.; and creatinine, 2.7 mg. per 100 cc. The icteric index was 10.1 units with a direct immediate positive van den Bergh.

A diagnosis of partial low intestinal obstruction and pulmonary congestion was made on admission, and treatment was at first directed towards the relieving of the intestinal obstruction. On March 20, the 3rd day after admission, the pulmonary findings became more pronounced and a diagnosis of pneumonia was made. On March 22, sulfapyridine therapy was started. The first dose of 1 gm. was given at 10 P.M. Thereafter, for the following 2 days, the drug was given in doses of 1 gm. 4 times a day, until a total of 8 gm. was consumed by the end of the 2d day. On March 25, the patient began to show an anemia and his skin took on a yellow hue.

Blood examination that day showed a marked drop of hemoglobin and red blood cells. On March 23, the first day after sulfapyridine administration, the hemoglobin was 80% and the erythrocyte count 4,000,000 per cc. On March 25, the hemoglobin fell to 58% and the erythrocytes to 2,750,000 per cc. Leukocyte count was 18,500 per c.mm. with 66% neutrophils, 2% metamyelocytes, 2% myelocytes and 30% lymphocytes. The erythrocytes showed marked anisocytosis, poikilocytosis and polychromasia, and contained 4% of nucleated forms. The urine became port-wine in color, was positive for blood and bile and contained 10% of packed coagulated erythrocytes.

Sulfapyridine was immediately discontinued and a continuous intravenous administration of 5% glucose and saline was begun. The *blood* on the following day, March 26, showed an icteric index of 20.5 and on March 27, it rose to 30.5, with an immediate direct positive van den Bergh. The blood glucose was 421 mg.; urea nitrogen, 222; non-protein nitrogen, 258; and creatinine 8.86 mg. %. The sulfapyridine content of the blood was 11.5 %. Leukocyte count was 26,000 per c.mm. with 65% neutrophils. The nucleated red blood cells increased to 8%. The temperature rose to 102.5° F. Pulse varied between 100 and 115 and respiration between 20 and 26 per minute. In spite of the discontinuation of the drug, the patient passed into a semi-stuporous state and his jaundice deepened. The following day, March 26, two transfusions of 250 cc. and 500 cc. of citrated blood, respectively, were given with no untoward effect. The following day, March 27, 9 days after admission, the uremic state deepened, and at 3 P.M., 65 hours after administration of sulfapyridine, he died. He had complete anuria for 36 hours preceding death.

Autopsy (3 hours after death) revealed the following pertinent findings: jaundice of skin, conjunctivæ and viscera; partial intestinal obstruction in the region of the ascending colon caused by a fibrous band; suppurative bronchitis, emphysema and basal atelectasis and sclerosis of coronary vessels. The *kidneys* were moderately enlarged. They weighed 175 gm. and measured about 12 by 5.5 by 3 cm. each. Capsule stripped with ease. Surface was finely granular. On section, throughout the cortex and medulla, and especially at the cortico-medullary junction, there were numerous brownish specks arranged in coalescent longitudinal streaks which made the outline of the rays prominent.

Microscopically, the most profound changes were found in the kidneys and the liver. The *kidneys* (Figs. 1–5) showed the majority of the tubules to be plugged with collections of golden-yellow or brownish pigment casts or hemosiderin-laden macrophages and clumps of granular "shadow" erythrocytes. This was mostly noted in Henle's loops, the recurring limbs and collecting tubules. In many tubules (Figs. 2, 3), the free and intra-

cellular pigment plugged and distended the lumens causing pressure atrophy and thinning of their lining epithelium. In a smaller number (Fig. 3), the tubular epithelium contained coarse brownish hemosiderin granules within the cytoplasm, and the cells, including the nuclei showed varying stages of degeneration. Beginning calcium deposition around few tubular casts were noted (Fig. 4). No evidence of tubular regeneration was found. The proximal portions of the tubules were widely dilated (Fig. 5.) The glomeruli showed relatively few changes compatible with those found in the aged. The *liver* (Fig. 6) showed patchy cytoplasmic vacuolization, with a tendency towards central localization. The Kupffer cells were increased in number and loaded with hemosiderin pigment. In the *spleen*, the reticulo-endothelial elements were very prominent and contained abundant hemosiderin pigment. The *bone marrow* showed a moderate degree of hyperplasia of the erythropoietic elements. *Prussian blue* stains demonstrated the presence of iron-containing pigment in the tubular casts, the Kupffer cells, the reticulo-endothelial elements of the spleen and, to a lesser degree, in the liver cells, and occasionally in the epithelium of the renal tubules.

Pathologic Diagnosis. Acute hemolytic anemia with hemosiderosis of the liver, spleen and kidneys; fatty degeneration of liver; widespread obstruction of renal tubules (mainly of Henle's loops, recurring limbs and collecting tubules), with secondary dilatation of proximal tubules and a moderate degree of tubular degeneration; moderate vascular nephrosclerosis; sclerosis of coronary vessels with patchy myocardial fibrosis; partial obstruction of ascending colon, caused by bands of adhesions, as a sequela of a prostatectomy operation; chronic suppurative bronchitis, pulmonary emphysema and bilateral basal atelectasis; moderate hyperplasia of the bone marrow.

Discussion. As far as we could ascertain, this appears to be the first reported fatal case of acute hemolytic anemia occurring during sulfapyridine treatment. In evaluating the exact cause of death in our case, we cannot lose sight of several contributory factors which were present. The co-existing partial and moderate intestinal obstruction and the senile, "decrescent" cardiovascular and renal lesions, though, *per se*, rather moderate, deserve special consideration. The intestinal obstruction was undoubtedly the cause of the moderate retention of the nitrogenous products in the blood on admission. However, after careful consideration of the gross and microscopic findings, we are prepared to relegate it to a minor place, especially since under treatment the signs of obstruction subsided considerably. On the other hand, the sudden and dramatic onset of the acute hemolytic anemia, with its resulting complete renal shut-down, as so clearly demonstrated by the tubular blockage, coupled with the severe liver damage, leave no doubt in our mind that it was sulfapyridine which was directly and mainly responsible

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LEGENDS FOR FIGS. 1, 2 AND 3.

FIG. 1.—Kidney showing plugging of tubules, mainly in the medulla and corticomedullary junction and secondary dilatation of the proximal portions of the tubules in the cortex. (× 12.5.)

FIG. 2.—Kidney showing plugging of tubules in the medulla with pigment casts and secondary pressure atrophy of the tubular epithelium. (× 100.)

FIG. 3.—Kidney showing in addition to plugging of lumen of tubules with hemoglobin pigment and shadow erythrocytes also hemosiderosis of the epithelium and various stages of necrosis of the epithelium proper. (× 300.)

FIG. 1

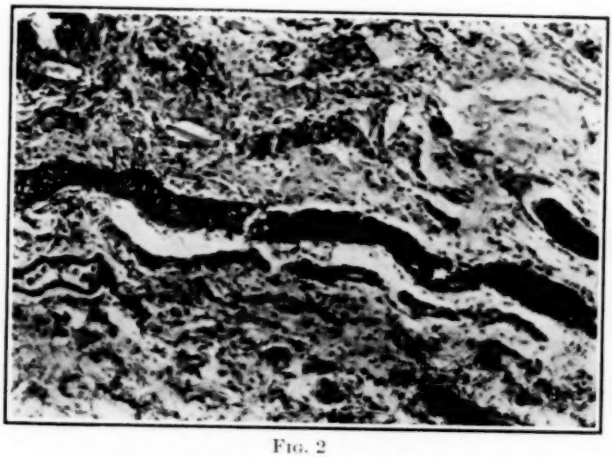

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

FIG. 4.—Calcium deposition on a pigment cast in one tubule. (× 450.)

FIG. 5.—Secondary dilatation of the proximal convoluted tubules in the cortex and a few plugged distal segments of the tubules. (× 450.)

FIG. 6.—The liver showing fatty degeneration with a tendency towards localization in the central part of the lobule. (× 25.)

FIG. 7.—The kidney after death due to transfusion with incompatible blood, showing plugging of tubules with hemoglobin pigment and secondary thinning of tubular epithelium. (× 450.)

for the death of the patient. The other factors enumerated above can, at most, be considered as contributory.

Reviewing the literature[5,8,9,11–13,15,18] on acute hemolytic anemia due to the closely allied drug, sulfanilamide, it is seen that, under prompt treatment, which consisted mainly of transfusion and immediate withdrawal of the drug, recovery took place in most of the patients.

There is only one recorded fatal case of acute hemolytic anemia caused by sulfanilamide (Wood[21]). A comparison of Wood's case with our own reveals several interesting similarities. The jaundice and anemia were noted in both cases on the third day of administration of the drug. The necropsy findings in Wood's patient are similar to ours, being mainly those of hemolytic anemia with hemosiderosis of liver, spleen and kidneys and blocking of the kidney tubules with hemoglobin casts. However, no stress is laid in Wood's report on the tubular obstruction, nor are there any blood chemistry figures available for study of the renal function.

It is interesting to note that the time interval of 3 days for the onset of the anemia in our case was about the same as that observed by Long.[11] He also found that this had no relation to the total amount, nor to the size of the individual doses of the drug administered.

The renal lesion and even the clinical features of our case bear a striking similarity to those seen in some of the patients who die as a result of transfusion with incompatible blood. The latter exhibit also all the features of true renal insufficiency with widespread changes in the renal tubules and hemosiderosis of the liver and the reticulo-endothelial system. They also show either a blocking of the distal segments of the tubules by pigment casts and secondary dilation of the proximal portions, or varying degrees of degenerative changes in the tubular epithelium itself.

An even more striking similarity with the anatomic findings in our case is seen in those of experimental renal insufficiency, as obtained by DeGowin and his associates.[4] The majority of their dogs, who died from renal insufficiency, as a result of injection with a solution of canine hemoglobin, showed pathologic lesions that are almost identical with those in our case, as well as in a number of reported deaths after transfusion and in one case after quinine administration. (Bordley;[3] Goldring and Graef;[6] Baker and Dodds;[2] Witts;[20] Shera;[16] Ravid* and Terplan and Javert.[19]) The main lesion in all these was obstruction of the tubules by hemoglobin pigment (Fig. 7). The other lesion, namely necrosis of the tubular epithelium, which in some transfusion cases, as well as in a number of DeGowin's dogs, was more prominent, was also seen in our case, but only to a lesser degree.

* Ravid, J. M.: A case of death following transfusion with incompatible blood, in a woman of 42 (unreported). Microphotographs of the kidney of this patient accompany text (Fig. 7).

We can, therefore, conclude from the anatomic lesion in our case, that the tubular obstruction and, to a lesser degree, the degenerative changes in the tubular epithelium, were the salient factors in the mechanism of renal insufficiency and uremia.

Recently, 3 cases of "hematuria, abdominal pain and nitrogen retention, following sulfapyridine administration" were reported by Southworth and Cooke.[17] In commenting on their cases, the authors state that "the mechanism underlying the development of these complications has not definitely been determined."

It seems to us, that in the light of the anatomic findings in our case, coupled with those in the case of Lawrence[10] in man, and in the experimental work of Oakley.[14] Antopol and Robinson[1] and Gross, Cooper and Lewis,[7] who saw the formation of uroliths after the administration of sulfapyridine and prontosil, the pathogenesis of the above 3 cases can also be reasonably explained. The symptomatology of these cases, not excluding the one that simulated acute appendicitis, was clearly of renal origin, as it was accompanied or immediately followed by gross hematuria. The pain was most probably due to distention of the kidney as a result of varying degrees of urinary retention or to impaction by a freshly formed calculus along the urinary tract.* The main lesion, most likely, was a partial blockage of the renal tubules by crystals or hemoglobin pigment, as a result of hemolysis and perhaps also a certain degree of degeneration of the tubular epithelium as a direct nephrotoxic action of the drug. This in turn must have resulted, first, in hematuria and, soon after, in suppression of urine, azotemia and uremic manifestations. The initiating process of this anatomic lesion, most likely, was either hemolysis or calculous impaction. At the same time, as seen in our case, in addition to the mechanical tubular blockage, another, so-called nephrotoxic factor, may have operated in their cases as well. It is also interesting to note that the dosage in these, as in other cases, was not a deciding factor. The total amount in all 3 cases was much higher than in ours (52 gm., 25 gm. and 10 gm. in authors' cases, as against 8 gm. in our case.)

Summary and Conclusions. 1, A case of fatal acute hemolytic anemia and uremia during the course of treatment with sulfapyridine, is reported here. It occurred on the third day of treatment, and only after a total of 8 gm. of the drug was consumed.

2. The salient pathologic lesions, directly caused by sulfapyridine were twofold: *first*, those secondary to the resultant hemoglobinemia and hemoglobinuria, viz., obstruction of renal tubules by hemoglobin pigment and its derivatives, and hemosiderosis of liver, kidney and the reticulo-endothelial system; and, *second*, those produced

* The fact that the Roentgen rays were "negative" does not exclude the presence of calculi because, as pointed out by Antopol and Robinson,[1] the uroliths consisting of the acetyl derivative of sulfapyridine permit penetration by Roentgen rays. It is only when calcium is deposited about these concrements which act as a nucleus that their shell may become Roentgen ray opaque.

as a direct action on the hepatic and renal epithelia proper. The latter lesion was minimal in this case.

3. The formation of urinary calculi must also be taken into account in the summation of the toxic effects of sulfapyridine on the urinary apparatus in general.

4. The clinico-pathologic picture, as induced by sulfapyridine, is not a syndrome *sui generis* for this drug alone, but appears to be analogous with those encountered in deaths after transfusion with incompatible blood, in blackwater fever, paroxysmal hemoglobinuria and poisoning with certain chemicals.

5. Careful daily observations of the patient, especially of the aged, with regard to the blood picture and the renal function, and the immediate discontinuance of the drug upon the detection of any deleterious effects on the kidneys and blood, should, among other things, be guiding principles in the treatment with sulfapyridine.

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PARENTERAL ADMINISTRATION OF SULFAPYRIDINE.

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IN the ordinary course of treatment of pneumococcic infections with sulfapyridine one frequently encounters situations which make the oral route of administration either impractical or impossible. Experience here and elsewhere has shown that nausea and vomiting may assume such troublesome, and sometimes even such serious

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proportions as to prevent effective use of the drug by mouth. Furthermore, special occasions arise in which it is difficult to effect satisfactory therapy *per os*. Among these situations may be: 1, the treatment of comatose patients; 2, the treatment of any urgent condition which requires an immediate, adequate, therapeutic blood level; and 3, the raising of the drug concentration in a localized area to a level equal to or higher than that in the blood, as, for instance, in the subarachnoid space of patients with meningitis.

A number of methods have been employed to obviate the above-mentioned difficulties. Almost every clinical investigator has mentioned some mode of administration which he believed would overcome the commonest of the ill-effects, namely, the nausea and vomiting. Early were mentioned the use of some soft food such as applesauce, of soda bicarbonate, or of milk. Later, rectal instillations of the drug were employed but without sufficient benefit to warrant their continued trial. Here we resorted to tablets coated with salol. In a few cases there was some slight alleviation of the gastric distress, but this improvement was hardly universal enough to be called satisfactory. Still later we resorted to the administration of 20 to 30 cc. of colloidal aluminum hydroxide just preceding each dose of pills. Again the adjuvant seemed to help the occasional patient but in many cases it only served to increase vomiting. Whitby[7] and later Hobson and McQuaide[1] suggested the use of an oily suspension of sulfapyridine for injection into those patients who, by virtue of their upset stomach or of their comatose condition, were unable to take the drug by mouth. Long[2] pointed out the objections and dangers of this method. Recently Marshall and Long[5] have advocated the use of the sodium salt of sulfapyridine by the intravenous route, in order to maintain a satisfactory blood concentration in the face of these obstacles.

With the exception of the sodium salt, with which we have had no experience, none of the suggested methods of altering the mode of administration to lessen the unpleasant side reactions has proven entirely satisfactory in our hands. In general, the incidence of nausea and vomiting does not seem to have been materially altered; the oft-mentioned "irregularity of absorption" does not seem to have been obviated and the patient does not seem to have been relieved of disturbing treatments. Comatose and other severely ill or unresponsive patients have still presented a definite therapeutic problem with respect to a quick attainment of an effective concentration of the drug in the blood. Furthermore, in the case of diabetics and cardiacs with pneumonia it has been almost imperative to find some method of giving the medication with a minimum of ill-effects.

Experimental. Early in our work with pneumonia patients we attempted to give sulfapyridine* by hypodermoclysis. A few ex-

* The sulfapyridine powder used was obtained through the courtesy of Merck & Co.

periments with animals served to show that, when the drug was introduced as a solution under the skin, there was no evidence of local tissue damage. We then observed that adequate blood levels could be maintained by the parenteral route alone; that the dosage required to produce a therapeutic effect was somewhat smaller than the usual oral dose. We therefore investigated the solubility of the pure powdered sulfapyridine, using several concentrations of the drug in a number of different solutions. The object throughout this work was to find a fluid suitable for parenteral administration which, at the same time, would carry more than the orthodox 1 gm. of sulfapyridine per liter. Added impetus was given this work by the successful treatment of 2 comatose cases of pneumococcus meningitis. This treatment was, of necessity, entirely by the parenteral paths for the first few days.

The results of our solubility studies on sulfapyridine are shown in Table 1.

TABLE 1.—SOLUBILITY OF SULFAPYRIDINE.

Amount of sulfapyd. added to 100 cc. of solvent, gm.	Amount of sulfapyridine found dissolved when analyzed.					
	Distilled water 20° C. = 6 hrs., gm.	Saline .085% 16° C. = 8 hrs., gm.	.85% saline 5% glucose 16° C. = 20 hrs., gm.	Saline .85% 37° C. 8 hrs., gm.	Glucose 5% 16° C. = 24 hrs., gm.	.85% saline 5% glucose 25° C. = 4 days, gm.
0.050	0.052	0.052	0.052	—	0.047	—
0.100	0.097	0.101	0.103	—	0.094	—
0.150	0.141	0.157	0.154	—	0.143	—
0.200	0.141	0.130	0.174	0.208	0.187	0 187
0.250	0.084	0.091	0.163	0.190	0.224	0.245
0.300	0.085	0.087	0.160	0.168	0.239	0.279

It will be seen from these observations that the solubility of sulfapyridine is a function of at least two different factors: 1, the type of solvent; and 2, the temperature of the solution. At first it was thought that the increased solubility in glucose might have been due to a combination of the drug with the sugar but determinations of the glucose content of the solutions before and after the addition of the drug failed to show any significant variation in glucose concentration. Furthermore, since the amounts of sulfapyridine recovered from the solutions analyzed approximated so closely those which had been introduced, it seemed evident that if any combination had been effected between the glucose and the sulfapyridine, it must have been so loose as to be unimportant.

For the administration of sulfapyridine parenterally it is clear from these solubility studies that a 0.15% solution in 0.85% saline or a 0.2% solution in 5% glucose solution or in equal parts of normal saline and 5% glucose solution is practical, and such has proved to be the case, provided the solutions are not allowed to cool below room temperature.

Methods. The method used in preparing these solutions for analyses was identical with that which we have been using in the preparation of the sulfapyridine for therapeutic purposes. A weighed amount of the powdered drug was poured into the desired volume of the liquid to be tested or used. This liquid had been brought to the boiling point just before the addition of the powder. The mixture was shaken vigorously until all the powder had gone into solution, usually for 1 or 2 minutes. (Occasionally it seemed expedient to heat the mixture again to the boiling point in order to facilitate complete solubility.) The solution was then allowed to cool for 6 to 8 hours or until it had reached room temperature. Portions of these mixtures were then analyzed for their sulfapyridine content. (Therapeutically the solutions were generally cooled fairly rapidly to the desired temperature.)

The determinations were made with a modification of the sulfanilamide method described by Marshall and Litchfield.[4]

1. To 20 cc. of a 1 to 20 dilution of oxalated blood in water are added 5 cc. of 15% trichloracetic acid.
2. To 1 cc. of a 1 to 8 dilution of the standard aqueous 10 mg.% solution of sulfapyridine are added 2 cc. 15% trichloracetic acid and 7 cc. distilled water. (This ultimate dilution contains 0.0125 mg. sulfapyridine and is suitable for use with blood levels of 1.5 to 4.5 mg.%. Other dilutions may be made by varying the volume of 1 to 8 dilute standard, adding the acid and then making the volume up to 10 cc.)
3. To 10 cc. of blood filtrate or acidified standard are added 1 cc. of fresh 0.1% sodium nitrite.
4. After 3 minutes add 1 cc. of the molar sodium acid phosphate buffer containing ammonium sulfamate.[4]
5. After 2 more minutes add 5 cc. of alcoholic dimethyl alpha naphthylamine (1 cc. of dimethyl naphthylamine in 250 cc. 95% ethyl alcohol).
6. Wait 10 minutes and then compare in a colorimeter.

The error in this method was determined to be between 2 and 4% when calibrated instruments were used and the fluids analyzed were diluted to contain no more than 15 mg.% of sulfapyridine.

The solutions were observed to assume a pale golden color upon standing. Whenever the sulfapyridine precipitated out, it seemed to carry more and more of the substance with it so that after a day or two a considerable clump of powder could be visualized on the bottom of the flasks. However, when solution was complete, the sulfapyridine remained dissolved for several days. Aërobic and anaërobic cultures of such fluids were found to be sterile.

Clinical Application. In the present series there are 43 cases which have received sulfapyridine parenterally, as shown in Table 2. In Table 3 are given the 16 cases which received sulfapyridine by the parenteral route exclusively except in 2 cases which received some oral treatment.

TABLE 2.—CASES TREATED WITH PARENTERAL SULFAPYRIDINE.

Intravenous alone	2
Intravenous plus subcutaneous	4
Subcutaneous alone	10
Subcutaneous supplementary to oral	27
Total	43

We have had no difficulty in maintaining adequate blood levels by this method even over periods of a week or more (Cases 99 and 97). Furthermore, we have had an opportunity to study the

favorable therapeutic response which may be attained by this method of administration (Cases 103, 109, 96; Charts 1–*A* and 1–*B*). In addition to the subcutaneous and intravenous routes, we have introduced the solution routinely into the subarachnoid space in our cases of meningitis, and in 2 cases of empyema we have replaced the empyema fluid by a slightly smaller quantity of sulfapyridine solution.

The fundamental plan of therapy has been to administer the drug to the extent of 6 gm. in the first 24 hours and then to maintain the blood concentration by means of 4 gm. daily thereafter. For this purpose in almost every case the sulfapyridine was made up in 2-gm. lots. Each 2-gm. portion of the drug was contained in 1500 cc. of normal saline, in 1 liter of 5% glucose, or 1 liter of aliquot portions of normal saline and 5% glucose. The desired

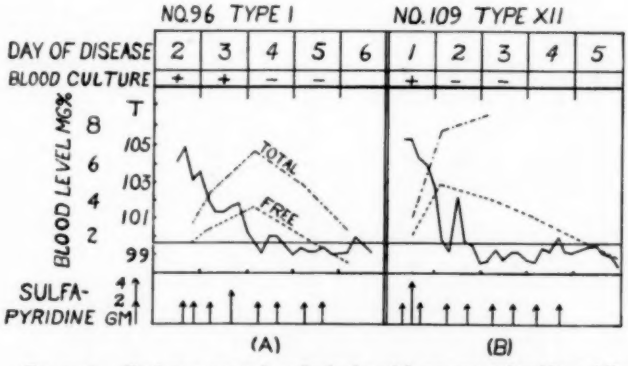

CHART 1.—Patients treated exclusively with parenteral sulfapyridine.

daily total could then be administered in as many individual treatments as seemed indicated. However, with each patient we tried to space the injections equally throughout the 24 hours; that is, every 8 hours (for 3 injections) or every 12 hours (for 2 treatments).

As can be readily seen in Chart 2–*A*, after an initial dose of 2 gm. the peak level of sulfapyridine in the circulation is attained at different times, depending on the route employed. The intravenous route gives its highest readings immediately after the solution has been introduced. On the other hand, hypodermoclysis affords its optimum level from 2 to 4 hours after the treatment has been completed. These levels compare more than favorably with the levels obtained by Schmidt and Hughes[6] and by Long[3] after an oral dose of 2 gm. or more of sulfapyridine.

It occurred to us that if the above observations were valid, it would be decidedly more efficacious to inaugurate the therapy in critical cases by a combination of these two approaches. Accordingly, 2 cases were treated as follows: After the type of pneumo-

coccus had been determined and the method of administration had been decided, a clysis of 2 gm. of sulfapyridine in 500 cc. of normal saline and 500 cc. of 5% glucose was started. While this solution was being absorbed, 2 gm. of sulfapyridine in 1000 cc. of 5% glucose were given intravenously. Chart 2–*A* shows that the sulfapyridine concentration in the blood is maintained at an almost constant level with the combined therapy. *A priori*, this procedure would certainly seem preferable to the others outlined.

However, in all fairness, it must be stated that, if it is desired to use one method exclusively, satisfactory blood levels may be maintained by giving the initial treatments oftener than every 12 hours. In this way accumulation of the drug occurs. Chart 2–*B* shows

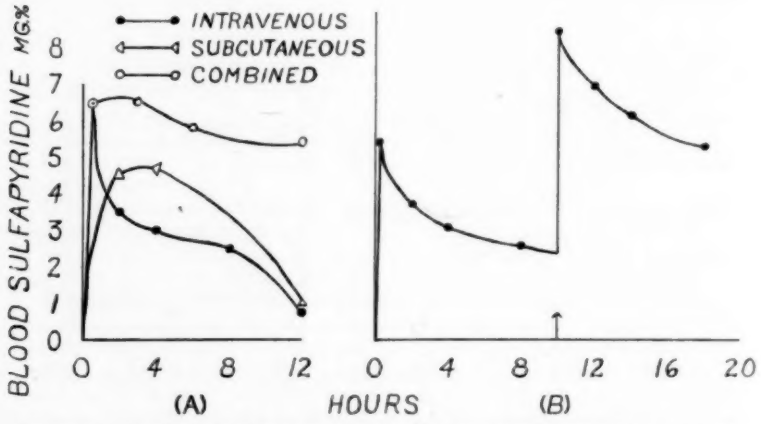

CHART 2.—Blood levels of patients treated with parenteral sulfapyridine. Initial dose, 2 gm.; 2 gm. by each route in combined treatment. (↑ indicates second dose of 2 gm. of sulfapyridine.)

such a procedure carried out by the intravenous route. In this case (No. 103) the second infusion of sulfapyridine was given about 10 hours after the first. Whereas, the figures for the 2-, 4- and 8-hour levels correspond very closely with those given for the single intravenous dose in Chart 2–*A*, the levels in the second 8-hour period were very much higher. Thereafter the blood concentration was readily maintained by injections every 12 hours (Table 3).

To date we have had only one local reaction following sulfapyridine given in the above described manner. One patient developed thrombophlebitis of the superficial thigh veins adjacent to the site of the injection. It seems likely that there was an error in technique to account for this accident, for nothing of a similar nature appeared in the opposite leg where concomitantly some of the same solution was being run in from the other arm of the "Y" tube of the same clysis set. Furthermore, we have observed no immediate

TABLE 3.—CASES TREATED WITH PARENTERAL SULFAPYRIDINE EXCLUSIVELY.*

TABLE 3.—CASES TREATED WITH PARENTERAL SULFAPYRIDINE EXCLUSIVELY.*

Case No.	Day of disease treatment started.	Route.	Dosage.	Blood levels†—days.					
				1st. (4 hr.)	2d.	3d.	4th.	5th.	6th.
103 (G. W.)	9th	I–v.	2 gm. 3 times in 1st 24 hrs. 2 gm. q. 12 hrs. thereafter	3.1, 3.4	5.3, 6.0	5.4, 7.4	6.6, 8.0	10.8, –	6.0, –
107 (J. M.)	3d	I–v.	2 gm. 3 times in 1st 24 hrs. 2 gm. q. 12 hrs. thereafter	3.0, –	2.8, 3.8	5.7, 7.4	–	6.1, –	–
146 (T. S.)	9th	I–v. + sci. oral	2 gm. i–v. and 2 gm. sci. simul. 8 hrs. later 2 gm. sci. 6 gm. q.d. orally thereafter	6.2, 6.6‡	4.9, 6.3	7.0, 8.9			
159 (H. D.)	1st	I–v. + sci.	2 gm. i–v. and 2 gm. sci. simul. 8 hrs. later 2 gm. sci. 2 gm. q. 12 hrs. sci. thereafter	6.9, 8.2‡	7.4, 9.8	6.5, –	5.5, –	trace, 5.5§	3.1, 8.1
109 (F. L.)	1st	I–v. + sci.	2 gm. i–v. on admission 8 hrs. later 4 gm. sci. 2 gm. 12 h. sci. thereafter	2.1, 3.1	4.9, 7.8	4.1, 8.6	3.1, –	–	
41 (J. L.)	2d	Sci.	2 gm. 3 times in 1st 24–48 hrs. 2 gm. q. 12 hr. thereafter	3.3, –	–	6.0, –	8.5, –	8.0, –	
65 (J. DeL.)	2d	Sci.	2 gm. 3 times in 1st 24–48 hrs. 2 gm. q. 12 hrs. thereafter	2.9, –	6.8, –	10.8, –	8.9, –	7.7, –	2.7, –
96 (L. S.)	2d	Sci.	2 gm. 3 times in 1st 24–48 hrs. 2 gm. q. 12 hrs. thereafter	1.6, 2.5	2.3, 4.2	3.5, 6.5	2.0, 4.8	0.6, 2.3	
101 (J. McL.)	2d	Sci.	2 gm. 3 times in 1st 24–48 hrs. 2 gm. q. 12 hrs. thereafter	3.0, 5.2	4.9, 8.0	9.4, 11.1	9.3, –	11.2, 12.6	10.2, –
15 (M. R.)	1st	Sci.	2 gm. q. 8 h. in 1st 24 hrs. 1 gm. q. 12 h. thereafter	3.1, –	7.1, –	6.0, –	3.9, –	–	4.6, –
99 (B. G.)	1st	Sci.	3 gm. q. 12 h. for 1st 48 hrs. 2 gm. q. 12 h. thereafter	–	5.1, 5.5	11.2, 13.3	8.9, 15.4	6.8, 8.9	4.2, 5.9
78 (W. B.)	2d	Sci. (+ serum)	2 gm. on 1st day 2 gm. q. 12 h. thereafter	–	–	7.8, –	4.3, –	3.9, –	
10 (M. S.)	2d	Sci. (+ serum)	2.5 gm. in 1st 24 hrs. 1–2 gm. q.d. thereafter	4.6, –	6.6, –	5.8, –	5.6, –	5.1, –	
71 (G. P.)	?	Sci.	2 gm. q.d.	–	1.0, –	2.3, –	4.4, –		
97 (M. D.)	–	Sci. + I–v.	6 gm. orally on 1st day 2 gm. parent. q. 12 h. thereafter	–	6.3, 9.4	11.9, 16.0	12.6, 16.2	7.9, 12.3	7.4, 13.2
36 (T. G.)	?	Sci.	2 gm. twice	–	3.1, –				

* Except in 2 cases which received some oral treatment.
† The first figure in each column represents free, the second figure total sulfapyridine.
‡ 3-hour levels. § Patient received only 1 gm. sulfapyridine on preceding day.

reactions such as chills or vascular collapse. Thus far no serious drug reactions have occurred in any of the cases that have been treated by the parenteral methods alone. At the moment it is not clear whether this is merely a fortuitous circumstance dependent on the small number of cases comprising this particular group. In general, it is fair to say that nausea and vomiting have been somewhat less severe, but by no means eliminated when the drug is administered parenterally. However, it ought to be stated that, in the few analyses which have been performed on the vomitus of patients who have received only parenteral sulfapyridine, the concentration of the drug in the vomitus has been appreciably higher than the level which was simultaneously observed in the blood. This fact would appear to leave somewhat in doubt the controversy as to whether the vomiting and nausea are of purely central or partially local origin.

Case Reports. CASE 99.—B. G., aged 41, female, white, admitted in state of acute alcoholism, March 19, 1939. Onset of pain in left lower chest with cough and fever 1 day before admission. Generalized convulsions on day of admission followed by a shaking chill. In addition to acute alcoholic delirium and gastritis, examination showed physical signs and Roentgen ray evidence of left lower lobe pneumonia. Type III pneumococcus was obtained from sputum. Sulfapyridine was given by clysis and temperature fell by crisis to normal after 32 hours of therapy. Forty-eight hours later temperature rose sharply to 104° F. and then fell gradually to normal over a space of 60 hours. Blood levels are tabulated in Table 3. Patient recovered. Sulfapyridine was stopped after 9 days.

CASE 97.—McL. D., aged 40, male, white; diagnosis of subacute bacterial endocarditis due to streptococcus was established March 17, 1939. Oral therapy with sulfapyridine produced intractable nausea and vomiting so the drug was administered by clysis and infusion. Temperature fell promptly to normal and remained flat for 6 days. Finally patient began to have gradually increasing amounts of fever so drug was stopped after 10 days and blood cultures became positive again.

CASE 109.—F. L., aged 39, male, white; late in evening of March 23, 1939, patient had sudden pain in left chest followed by cough, pink sputum and a mild chill. On admission 10 hours later, patient appeared prostrated and critically ill. Type XII pneumococcus was obtained from sputum and blood culture. Patient was put in an oxygen tent and the first dose of sulfapyridine was given intravenously. Subsequent doses were by clysis. Temperature fell by crisis in 24 hours. Blood cultures taken after therapy was started were sterile and patient made an uneventful recovery. Sulfapyridine was given for 4 days (Chart 1–*B*).

CASE 96.—L. S., aged 40, male, white; on March 16, 1939, patient had severe chills, fever and pain in right lower chest. He was admitted the next day at which time, in addition to moderately severe diabetes mellitus, signs of right middle lobe consolidation were discovered. Type I pneumococcus was isolated from sputum. Sulfapyridine was administered entirely by clysis. Blood culture was positive on admission and remained positive for 1 day after inauguration of drug therapy but temperature fell to normal in 32 hours and patient improved markedly. Clyses of sulfapyridine were given for 5 days and diabetes was readily controlled by insulin and diet (Chart 1–*A*).

CASE 103.—G. W., aged 59, male, white; on March 20, the seventh day after the diagnosis of pneumonia had been made, blood culture became

positive for Type V pneumococcus, about 150 col/cc. Up to this time several attempts to type a pneumococcus from the sputum had yielded only a very heavy growth of hemolytic Staphylococcus aureus. Patient appeared almost moribund and W.B.C. had dropped from 15,000 to 4800 in 3 days. Intravenous therapy was instituted for 6 days. Blood cultures became negative. W.B.C. rose and patient began to improve after about 48 hours. Patient later developed a lung abscess from which hemolytic Staphylococcus aureus and a few Type V pneumococci were obtained. Recovery has been slow.

Summary. We have presented a method of administering sulfapyridine parenterally which we deem quite satisfactory. One disadvantage of the method might appear to be the relatively large volume of fluid which must be used in order to put the desired quantity of sulfapyridine into solution. On the other hand, we have demonstrated by actual trial that such volumes may be given safely to almost any case when necessary. The method will maintain adequate therapeutic blood levels. It will provide adequate fluids, salt and glucose at a time when they are often needed either because of persisting vomiting or inadequate intake in delirious or comatose patients. It can be given without fear of slough or other reactions such as may follow the use of the extremely alkaline sodium sulfapyridine. Furthermore, it may be given into any part of the body where needed, such as the veins, the subcutaneous tissue, the pleural cavities or the subarachnoid space.

We are indebted to Miss Barbara Gallup for assistance in determining blood concentrations of sulfapyridine.

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OBSERVATIONS ON THE PHARMACOLOGY AND TOXICOLOGY OF SULFATHIAZOLE IN MAN.

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Sulfathiazole,† 2-(p-aminobenzenesulfonamido) thiazole, synthesized by Fosbinder and Walter[4] and Lott and Bergeim,[7] is the thiazole analogue of sulfapyridine. The report of Van Dyke, Greep,

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† We are indebted to Dr. George A. Harrop, Squibb Institute for Medical Research, New Brunswick, N. J., for the sulfathiazole used in these studies.

Rake, and McKee[15] showed that sulfathiazole is more rapidly metabolized by mice, rats, and monkeys, and undergoes less conjugation in rats and monkeys than sulfapyridine. These workers found that the drug administered with the food of mice was no more toxic than sulfapyridine when the dose was kept at therapeutic levels. Furthermore, sulfapyridine given to rats and monkeys for 14 to 57 days was found to be more toxic than sulfathiazole. When the sodium salts of the two drugs were used, sulfathiazole appeared to have about 65% of the toxicity of sulfapyridine for LD50. The principal toxic effect was the occurrence of albuminuria and hematuria in some animals.

The therapeutic effectiveness of sulfathiazole against experimental infection has been investigated by McKee, Rake, Greep, and Van Dyke.[8] If sulfathiazole or sulfapyridine sufficient to make 1% of the diet was fed to mice infected with pneumococcus (9 types), meningococcus, hemolytic streptococcus, or the agent of lymphogranuloma venereum, the therapeutic effect of sulfathiazole was equal to that of sulfapyridine. Further studies by this group as well as by Barlow and Homburger[2] suggested that sulfathiazole was more effective than was sulfapyridine against staphylococci.

The present report offers certain observations dealing with the pharmacology and toxicology of sulfathiazole in humans. Two groups of experiments were conducted, those in which a single dose of the drug was administered orally, intravenously, or rectally, and those in which multiple doses were administered over a period of days.

Methods and Material. Sulfathiazole was determined by the method of Marshall and Litchfield,[9] hemoglobin by the method of Sanford, Sheard, and Osterberg,[11] and urea in blood and urine by the method of Karr.[5] Urea clearance was calculated on the basis of 24-hour collections of urine. Preliminary trials demonstrated that sulfathiazole added to blood could be determined satisfactorily by the Marshall and Litchfield method. Slight losses, not exceeding 5%, occurred during the determination of total sulfathiazole.

The effects of the administration of single doses were studied in a group of 9 patients. Five (Cases 5 to 9) were convalescing from pneumonia but had been afebrile at least 7 days and had received no sulfonamide medication for at least 5 days. Two (Cases 1 and 2) were suffering from skin lesions of a moderate character (varicose ulcer and neurodermatitis) and 2 (Cases 3 and 4) were awaiting discharge from the hospital following uncomplicated herniorrhaphy.

The effects of continued administration of the drug was studied in 14 patients suffering from pneumonia; 83 patients undergoing treatment with sulfathiazole have been observed for possible toxic manifestations.

Results. 1. *Oral Administration.* Each of 6 subjects was given a single oral dose of 3 gm. of sulfathiazole shortly before a breakfast consisting of cereal, bread, milk, and canned fruit. It is evident from Table 1 that absorption from the gastro-intestinal tract was rapid. Within 1 hour, the concentration of free sulfathiazole in the

TABLE 1.—THE FATE OF SULFATHIAZOLE ADMINISTERED BY VARIOUS ROUTES.

Patient.	Date.	Drug. gm.	Hours after admin.	Blood.			Urine.				
				Hb, gm. per 100 cc.	Sulfathiazole.		Sulfathiazole.		Excreted. gm.	Volume. cc.	
					Free.	Total.	Free.	Total.			
					Mg. per 100 cc.						
	1939										
1. A. M. . . .	Dec. 19	3	0								
		(oral)	1	9.3	1.5	2.0					
			2	8.5	1.5	2.0					
			4	7.3	2.3	3.6	68	96	.188	196	
			6	8.5	3.5	3.5					
			8	9.3	4.6	4.7	225	235	.550	234	
	20	..	24	9.6	1.2	1.2	65	115	1.010	870	
2. B. R. . . .	19	3	0								
		(oral)	1	12.0	1.2	1.2					
			2	12.4	4.8	5.3					
			4	11.5	4.5	4.8	125	150	.525	350	
			6	12.0	3.4	4.0					
			8	12.0	3.2	3.3	175	200	.060	30	
	20	..	24	12.0	1.2	1.3	63	85	1.270	1490	
	1940										
3. C. C. . . .	Jan. 4	3	0								
		(oral)	1	14.8	5.0	5.2					
			2.5	14.8	5.0	5.6					
			4	14.0	4.0	4.7	180	192	.332	172	Crystals
			6	14.0	2.6	3.8					
			8	13.6	2.0	3.2	295	350	.395	113	Crystals
	5	..	24	14.0	1.2	1.5	180	275	1.790	650	Crystals
4. S. W. . . .	4	3	0								
		(oral)	1	14.8	5.0	5.0					
			2.5	14.8	6.3	8.5					
			4	14.8	5.7	5.9	62	62	.068	108	
			6	14.4	3.6	4.9					
			8	14.4	1.0	2.4	562	637	1.340	210	
	5	..	24	14.4	1.4	1.5	100	110	1.400	1275	
5. A. W. . . .	23	3	0								
		(oral)	1	13.2	4.5	5.0					
			2	13.2	5.5	6.5					
			4	13.2	4.7	6.0	135	162	.814	300	
			6	..	4.2	5.7					
			8	13.2	2.0	2.7	185	282	.764	270	
	24	..	24	13.2	0.0	0.0	75	135	1.130	825	
6. G. B. . . .	23	3	0								
		(oral)	1	14.4	2.3	3.0					
			2	14.4	5.0	6.4					
			4	14.4	4.4	6.4	25	32	.228	70	
			6	14.4	2.5	4.4					
			8	14.4	2.1	3.2	182	270	1.180	435	
	24	..	24	14.4	0.0	0.0	67	142	1.330	930	
7. C. P. . . .	10	Before;	..	13.6	0.0	0.0					
		3.4	0								
		intra-	0.25	13.2	8.8	9.8					
		ven.	0.5	12.7	7.3	8.0					
		as	1	12.7	5.5	6.5					
		sodium	2	12.4	4.3	5.0					
		salt	4	11.1	3.3	4.0	475	562	1.460	260	
			8	12.4	2.0	2.0	112	142	.968	680	
	11	..	24	12.7	0.0	0.0	37	55	.666	1210	
8. A. L. . . .	10	Before;	..	11.1	0.0	0.0					
		3.4	0								
		intra-	0.25	11.1	15.0	17.0					
		ven.	0.5	11.1	13.5	14.2					
		as	1	10.4	10.6	11.3					
		sodium	2	10.4	8.1	9.2					
		salt	4	10.4	6.3	7.2	465	500	.830	166	
			8	10.4	3.3	4.4	317	427	.720	168	
	11	..	24	11.1	0.7	1.2	167	240	1.560	650	
9. R. U. . . .	30	3	0								
		rectal	1	15.6	0.7	1.0					
		as	2	15.6	1.0	1.3					
		sodium	4	15.6	1.0	1.4	35	42	.128	300	
		salt	6	15.2	0.6	1.0					
			8	15.2	0.4	0.7	25	35	.088	250	
	31	..	24	15.2	0.0	0.0	15	25	.161	645	

blood reached or exceeded 4.5 mg. per 100 cc. in 3 of 6 subjects. In 5, the blood level continued to rise and reached a maximum value at the end of the 2-hour period. Concentrations remained above 4 mg. per 100 cc. for 4 to 6 hours. One patient (Case 1), aged 58, malnourished, and suffering from a varicose ulcer of the leg, differed from the remainder of the group. A delayed rise to a maximum at 8 hours suggested impaired ability to absorb the drug. However, the maximal concentration at this time was almost the same as in the more nearly normal subjects.

In this group of 6 the highest concentration of conjugated sulfathiazole in blood was 2.2 mg. per 100 cc. with a total sulfathiazole of 8.5 mg. per 100 cc. (Case 5). A few samples of blood and urine, especially those taken soon after the drug was given, contained none of the conjugated form.

Excretion in urine accounted for 84 to 93% of the ingested sulfathiazole within 24 hours in 4 subjects in the group (Table 1). Two others excreted about 60% in 24 hours. One of the latter was the patient (Case 1) showing delayed absorption of the drug. Crystals containing sulfathiazole and of acetylsulfathiazole precipitated from the urine of Case 3. Other patients have failed to show crystals in the urine, although higher concentrations of both forms often have been observed.

2. *Intravenous Administration.* Intravenous injection of sodium sulfathiazole into 2 patients (convalescent from pneumonia) gave recoveries of 96 and 97% of the drug in the urine in 24 hours (Table 1). This yield is within the experimental error of the method employed for sulfathiazole determination, so that only traces, if any, of the drug failed to be excreted in the urine.

Changes in concentration of hemoglobin were significant in only one subject after sulfathiazole was given by the oral route and in one following intravenous administration of sodium sulfathiazole. Presumably such changes reflect alterations in blood hydration, although confirmatory evidence is lacking.

3. *Rectal Administration.* Absorption of sodium sulfathiazole administered by rectum was slow and far from complete. Only slightly more than 10% of a 3 gm. dose was found in the urine within 24 hours (Table 1).

4. *Effect of Continued Administration.* In Table 2 are shown representative blood and urine studies of patients suffering from pneumonia who were receiving treatment with sulfathiazole. One or more specimens of blood and 24-hour collections of urine, when obtainable, were analyzed daily for 1 to 12 days. Following an initial administration of 3 gm., 1 gm. of sulfathiazole was given at 4-hour intervals. (Certain patients received a second 3 gm. dose 4 hours after the first.) Blood specimens were collected at various intervals. In 60 patients, including those in Table 2, concentrations of free sulfathiazole in blood during treatment varied from 1.2 to 19 mg.

TABLE 2.—EFFECT OF CONTINUED ORAL ADMINISTRATION OF SULFATHIAZOLE.

Patient.	Date.	Drug, gm.	Blood.					Urine.				
			Time of day.	Hb, gm. per 100 cc.	Urea N.	Sulfathiazole.		Sulfathiazole.		Volume, cc	Sp. gr.	Urea clearance, % of normal.
						Free.	Total.	Free.	Total.			
						Mg. per 100 cc.						
	1939											
10. J. B.	Dec. 12	6	11.00 A.M.	12.0	7	6.4	7.5	162	175	1285	1.021	102
			4.30 P.M.	12.0	..	3.0	3.4					
11. W. M.	11	4	7.30	15.6	..	2.2	3.9					
	12	6	11.00 A.M.	13.6	7	6.0	7.2	145	160	2775	1.018	97
			12.30 P.M.	15.2	..	3.1	4.0					
			4.30	12.0	..	3.4	3.8					
	13	6	11.00 A.M.	15.2	..	5.5	5.7					
			5.00 P.M.	12.4	8	3.2	4.5	225	267	1220	1.014	54
	14	6	11.30 A.M.	15.2	6	3.1	4.9	250	400	1810	1.013	125
	15	6	11.00	12.7	..	4.0	4.2	107	145	1725	1.016	
	16	0	....	..	..	..	..	38	40	1680	1.015	
12. M. B.	11	6										
	12	6	11.00	12.0	..	11.4	14.3					
			12.30 P.M.	14.4	8	8.5	12.7	275	400	1000	1.018	130
	13	6	....	..	..	..	..	435	575	330*	1.020	
	14	6	....	..	..	..	..	625	875	150*	1.023	
	15	6	1.00	12.0	..	3.0	4.0					
13. F. H.	14	2	....	..	..	..	..	325	425	525	1.024	
	15	6	1.00	13.2	7	4.9	5.7	240	350	1060	1.014	48
	16	6	....	13.2	6	3.7	4.6	220	300	1440	1.012	124
	17	6	....	..	..	..	..	165	248	1695	1.011	
	18	6	....	..	..	..	..	185	215	1115	1.012	
	19	6	....	12.7	..	3.5	4.4					
14. J. C.	Nov. 21	6	10.15 A.M.	..	..	4.0						
			12.00 P.M.	..	..	3.0						
	22	7	....	..	15	3.0	3.6	156	223	800	1.023	128
15. M. J.	29	5	9.30 A.M.	..	..	3.7	4.1					
			11.00	..	..	5.1	6.5					
	30	6										
	Dec. 1	6	12.00 P.M.	7.7	8	4.0	5.5	257	305	1610	1.011	51
	2	6	10.00 A.M.	6.9	7	4.5	5.2	162	212	2030	1.012	42
16. W. W.	Nov. 30	6	11.30 A.M.	..	..	3.6	3.6	240	265	1670	1.023	50
	Dec. 1	6	....	..	..	3.4	3.7	350	410	1575	1.022	
	2	6	10.00	..	..	3.4	4.7	190	212	1850	1.012	
	3	6	....	..	..	..	..	190	212	4100	1.011	68
	4	6	....	18.4	10							
	5	0	....	14.0	..	0.5	0.8					
	6	6										
	7	6	....	14.8	9	2.0	3.6	310	365	1735	1.015	150
	8	6	....	..	..	..	..	265	352	1065	1.020	
	9–10	12										
	11	0	....	15.6	..	2.6	3.5					
17. T. Q.	5	4	3.00 P.M.	10.8	11	5.5	6.0					
	6	6	7.00	..	..	4.8	5.5					
			2.30	12.0	8	5.2	5.4	415	737	450	1.021	101
			5.30	..	..	4.6	4.7					
	7	6	11.00 A.M.	11.1	8	4.2	4.8	306	360	*	1.013	
			4.30 P.M.	..	..	4.1	4.3					
	8	4	11.30 A.M.	10.4	..	4.5	5.8					
	9	6	11.30	10.0	8	4.4	5.6	257	357	2475	1.010	39
	10	0	10.30	10.4	7	1.0	1.4	60	79	3360	1.009	34
18. F. M.	19	2										
	20	6	11.00 A.M.	12.7	20	4.2	4.2					
	21	6	12.00 P.M.	11.1	20	3.6	4.5	405	540	1300	1.019	75
	22	6	4.00	12.7	12	4.0	4.2	323	415	1200	1.020	33
	23	6	1.30	12.7	8	3.6	4.1	213	243	1490	1.016	44
	24	6	2.00	..	..	3.1	3.4	270	317	1490	1.017	60
	25	0	....	12.7	..	0.2	0.7					

TABLE 2.—EFFECT OF CONTINUED ORAL ADMINISTRATION OF SULFATHIAZOLE —*Continued.*

Patient.	Date.	Drug, gm.	Blood.					Urine.				
			Time of day.	Hb, gm. per 100 cc.	Urea N.	Sulfathiazole.		Sulfathiazole.		Volume, cc.	Sp. gr.	Urea clearance, % of normal.
						Free.	Total.	Free.	Total.			
						Mg. per 100 cc.						
	1939											
19. E. H.	17	5	....	13.6	..	4.8	5.5					
	18	6	11.00 A.M.	13.2	7	3.1	4.1	300	375	1330	1.022	
	19	6	10.30	17.2	6	4.2	5.0	165	200	1375	1.012	22
	20	6	11.00	14.0	20	4.6	5.4	137	192	2700	1.011	197
	21	0	12.00 P.M.	15.2	20	0.3	1.0	135	187	3360	1.010	211
	22	0	....	..	..	..	..	55	78	2495	1.010	68
20. J. D.	Dec. 17	1										
	18	6	11.00 A.M.	12.7	..	3.6	4.1					
	19	6	10.30	13.2	..	3.3	4.0	200	235	*	1.024	
	20	6	....	..	..	..	..	410	555	670	1.024	
	21	6	....	..	..	..	..	332	485	1630	1.023	
	22	6	4.00 P.M.	15.2	12	2.7	4.0	200	260	1640	1.023	44
	23	6	1.30	15.2	11	2.5	3.2	205	247	1180	1.026	68
	24	6	2.00	13.6	11	2.5	2.8	307	350	1380	1.029	152
	25	0	....	16.0	..	1.0	1.2					
	1940											
21. E. R.	Jan. 11	8	11.00 P.M.	..	..	4.5	6.5	312	500	635	1.025	
	12	6	....	12.7	16	4.0	5.7	367	575	760	1.023	85
	13	6	1.00	12.4	12	4.6	6.6	392	625	830	1.025	60
	14	5	....	..	..	..	..	220	365	590	1.023	
	15	0	5.00	13.6	15	0.0	0.0					
22. T. B.	14	7	....	..	..	..	..	287	450	630*	1.029	
	15	6	....	12.4	21	8.0	10.4	387	485	100	1.023	38
			5.00 P.M.	..	15	3.1	6.2					
	16	6	....	..	..	..	..	412	637	420	1.026	71
	17	6	3.20 P.M.	12.0	8	3.6	5.5	285	375	430	1.026	73
	18	0	5.00	11.5	10	0.0	0.4					
23. A. H.	16	9	....	..	..	..	..	200	362	200*	1.019	
	17	6	3.30 P.M.	10.4	18	8.0	13.8	192	257	305	1.020	29
	18	6	5.00	11.1	18	5.4	8.2	275	275	570	1.022	42
	19	6	4.00	12.7	15	2.8	5.0					
	20	6	8.30	11.1	11	2.7	3.4					

* Not a 24-hour specimen.

per 100 cc., averaging 5.45 mg. per 100 cc. ± 3.42 (standard deviation). Conjugated sulfathiazole ranged from 0 to 9.7 mg. per 100 cc. with an average concentration of 1.73 mg. per 100 cc. ± 1.87 (standard deviation). Concentrations exceeding 4 mg. per 100 cc. of conjugated sulfathiazole were observed in only 5 patients, and in each case high values for this fraction were associated with severe illness. This correlation is of interest in connection with the suggestion of Wilson, Kaely, and Cullen[16] that a sudden rise in conjugated sulfapyridine may serve as a warning of danger. Brown, Thornton, and Wilson[3] also have commented on the serious implications of elevated levels of conjugated sulfapyridine.

Although Van Dyke and his associates found that sulfathiazole was not conjugated to the same extent as sulfapyridine by animals, we are not certain that this is true in man. Comparison with the

results of Long and Feinstone[6] and of Stokinger[13] for sulfapyridine failed to show much difference in response to single doses given by the oral or intravenous routes. There was, perhaps, with continued administration of sulfathiazole less of the tendency toward the accumulation of conjugated at the expense of the free compound that often accompanies medication with sulfapyridine. Because of the great importance of the condition of the patient in determining the extent of conjugation, comparisons must be made simultaneously under identical conditions. Further studies along these lines are in progress.

Absorption of the drug proceeded nearly as rapidly in patients ill from pneumonia as in the convalescent patients listed in Table 1, although exceptions were encountered. However, only 60 to 80% of the administered drug was recovered in the urine of these patients, somewhat less than in most controls.

5. *Effect on Kidney.* Inspection of Table 2 indicates that kidney function was temporarily impaired to a varying degree in nearly all of the patients. Oliguria was observed in 3 of 14, and hyposthenuria in 6 of 14 of the patients. Urea clearance was diminished in 9 of 14 patients during sulfathiazole therapy to 50% or less of the mean normal. The change, however, was transitory, and return to normal kidney function occurred in all except 4 patients during continuing sulfathiazole administration. In 2, the persisting low urea clearance could be explained by accompanying oliguria. That the comparatively moderate impairment of kidney function should be attributed to sulfathiazole seems doubtful, since other important contributing factors are known to be operative under these conditions.

Rough calculations of the clearances of free and conjugated sulfathiazole from data in Tables 1 and 2 showed little difference between the two forms. The average clearances of 8 patients presenting sufficiently high concentrations of the conjugated form to enable accurate measurement were 71 cc. of blood cleared per minute of the free, and 78 cc. per minute of the conjugated sulfathiazole. For sulfanilamide and sulfapyridine, Stewart, Rourke, and Allen[12] and Taylor and associates[14] found much lower values for the free forms, whereas clearances of the conjugated drug approximated those we have found for sulfathiazole. These observations imply that sulfathiazole is not reabsorbed as readily from the kidney tubules as are sulfanilamide and sulfapyridine.

In the presence of impaired kidney function, clearances of both free and conjugated sulfathiazole were lowered, the conjugated to a greater extent than the free.

Occasional specimens of urine of sulfathiazole-treated patients submitted for examination were opalescent and gave the impression that chylous urine was being secreted. Investigation showed this to be caused by a reaction between sulfathiazole or a derivative of

sulfathiazole and formaldehyde present as a preservative in one type of container used in this hospital.

6. *Toxic Effects.* Eighty-three patients suffering from pneumonia, including those listed in Table 2, have been observed for possible toxic manifestations. The incidence of untoward effects is shown in Table 3. In 4 patients nausea and vomiting were present before the start of sulfathiazole treatment, and in 2 these complaints followed the intravenous administration of sulfathiazole sodium. Unlike the severe and persistent vomiting caused by sulfapyridine, the vomiting induced by sulfathiazole was mild and infrequent and was marked by absence of severe nausea between attacks. In no case did nausea and vomiting necessitate discontinuance of therapy. Tabulated reports of the toxic effects of sulfapyridine recently published by some of the writers[10] and by Abernethy, Dowling, and Hartman[1] show a considerably higher incidence of vomiting when sulfapyridine was used.

TABLE 3.—TOXIC REACTIONS TO SULFATHIAZOLE (83 PATIENTS).

	Incidence.
Nausea and vomiting:	
Vomiting, 1 or 2 times	7
more than 2 times	1
Vomited before drug and continued after	4
Hematuria, microscopic	5
gross	0
Dermatitis	3
Psychosis*	3
Leukopenia	0
Acute hemolytic anemia	0
Drug fever	0
Agranulocytosis	0

* Two alcoholic patients, 1 senile hypertensive patient.

Dermatitis, apparently caused by the drug, was observed in 3 patients. Mental confusion was encountered in several patients, but in view of the severity of their illness, it was difficult to evaluate properly the rôle of the drug as the causative agent. Psychosis in 3 patients is to be explained by the presence of chronic alcoholism in 2 and advanced arteriosclerosis in 1. Repeated blood counts and hemoglobin determinations made during the course of drug administration failed to show evidence of any unusual depression in the number of the white or red blood cells or in concentration of hemoglobin. Urine studies showed no marked deviation from the usual findings for patients with severe febrile illness.

As already mentioned, crystals containing the drug were observed in the urine of 1 patient. However, sulfathiazole is much more soluble than sulfapyridine, so that hazard from precipitation in the urinary tract should be less when the former is used. Sulfathiazole did not seem to aggravate or increase the frequency of the cyanosis of pneumonia.

Comment. In case of absorption and excretion sulfathiazole resembles sulfanilamide. Because sulfathiazole is absorbed more

readily from the gastro-intestinal tract and excreted more rapidly than sulfapyridine, its use therapeutically is more easily controlled, an advantage that at times may be of decided importance. It is evident that sulfathiazole shows certain of the toxic effects described for other therapeutically active sulfonamido compounds. The toxic effects do not seem to offer any serious barrier to its use in treatment, and indeed in many ways toxicity appears to be less than that of sulfapyridine.

Summary. 1. The behavior of sulfathiazole has been investigated in 9 convalescent patients serving as controls and in 83 patients suffering from pneumonia.

2. Sulfathiazole is rapidly absorbed from the gastro-intestinal tract and is rapidly excreted in the urine. Following intravenous administration of the sodium salt, recovery of sulfathiazole in the urine is practically quantitative. It is not readily absorbed from the rectum.

3. The proportion of the drug conjugated is low in most individuals.

4. Effects observed on renal function and the hemopoietic system were transitory and not pronounced.

5. Vomiting occurred in about 10% of the patients treated with sulfathiazole, but was never severe enough to interfere with the use of the drug.

6. The incidence of other toxic reactions was small.

We wish to acknowledge the assistance of Miss Shlomith Bethlahmy, detailed for this and related studies on pneumonia by the Pennsylvania State Department of Health, Pneumonia Control.

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BOOK REVIEWS AND NOTICES

CIRCULATORY DISEASES OF THE EXTREMITIES. By JOHN HOMANS, M.D., Clinical Professor of Surgery, Harvard Medical School. Pp. 330; 29 text illustrations and 13 plates. New York: The Macmillan Company, 1939. Price, $4.50.

IN the last few years surgeons have interested themselves more and more in the circulatory diseases of the extremities. As the studies on the physiology of the sympathetic nervous system, lymphatics and the blood-vessels have been extended, a great deal of material relevant to clinical symptoms resulting from disorders of the circulation has accumulated. This material has been weighed and evaluated and the result is presented in an organized manner by the author.

Arterial diseases, arteriosclerotic deficiency, thrombo-angiitis obliterans, spasm of the arteries and embolism are clearly discussed so that there should be little confusion in the minds of those who carefully study these chapters on the differentiation of these disorders. In the same way he has presented diseases of the veins and lymphatics.

While surgical treatment is included the main object of the book is not to give detailed surgical technic but to present a complete picture of the diseases discussed so that this volume is of equal importance to the physician and surgeon.

The Reviewer feels that it is almost an impertinence to ask for anything more than this excellent work; but he believes that a general bibliography would be more helpful than the chapter references, where there is much overlapping of references. He would also suggest a more complete index. In the second edition there should also be a discussion of heparin as a therapeutic agent.

This book is of great importance and is recommended to every practitioner of medicine. I. R.

OUTLINE OF PHYSIOLOGY. By WILLIAM R. AMBERSON, PH.D., Professor of Physiology, University of Maryland, and DIETRICH C. SMITH, PH.D., Associate Professor of Physiology, University of Maryland. Pp. 412 (double column); 177 illustrations (15 in color), by NORRIS JONES, Instructor in Scientific Illustrating, Swarthmore College. Baltimore: The Williams & Wilkins Company, 1939. Price, $4.00.

THIS text written by two teachers of the University of Maryland has recently been issued. It is admirably adapted for dental students, advanced college students and nurses, and might be used for collateral reading by students of medicine. Dr. Amberson has drawn upon his experience as a teacher in planning this text. At the outset some subjects, of a preliminary nature, are briefly but clearly discussed, the historical advances of the subject are briefly included, and the several phases of physiology are added. Although much of the detailed statements of larger texts are omitted, the basic facts of physiology are thoroughly discussed. Very few of the illustrations are from older texts. The illustrations and diagrams serve a definite purpose in following the descriptions in the text. It is an admirable text and the authors are to be congratulated upon its planning and completion. B. McG.

PHYSIOLOGICAL CHEMISTRY. A Textbook for Students. By ALBERT P. MATHEWS, PH.D., Andrew Carnegie Professor of Biochemistry, The University of Cincinnati. Pp. 1488; 113 illustrations. Sixth edition. Baltimore: The Williams & Wilkins Company, 1939. Price, $8.00.

THE new edition of this well-known text represents a serious attempt to bring the subject up-to-date since the publication of the previous edition in 1930.

The text is very readable and follows the author's bent in presenting the subjects with due emphasis upon historical development, and a characteristic tendency for a very personal imaginative appraisal of certain facts, their interrelationship and physiologic significance. Many teachers and students of the subject may prefer a much shorter text, and one much more objective in presentation. However, the choice of text is to an extent a matter of personal predilection, and some students no doubt are stimulated by an enthusiastic manner of presenting a subject.

The chapters upon Hormones, Vitamins and Mineral Metabolism are excellent. The love of the author for such terms as "cryptorrhetic" is excusable, but it denotes a tendency to pedantry which should not be encouraged in the student. In a sincere desire for historical approach, the author has erred in the use of relatively unreliable old data, when new precisely quantitative data could have been employed. For example, the absorption spectra of hemoglobin, as determined by the crude methods of Dhéré and Rollet (pages 781 to 784), may be cited. Such statements as "methemoglobin is a parahematin" (page 1004, footnote) is certainly misleading. The discussion of oxidation-reduction processes in the organism could have been improved materially if it were treated under a separate heading and the material upon the various oxidation-reduction enzymes had been included in this section. D. D.

SYNOPSIS OF PEDIATRICS. By JOHN ZAHORSKY, A.B., M.D., F.A.C.P., Professor of Pediatrics and Director of the Department of Pediatrics, St. Louis University School of Medicine, and Pediatrician-in-Chief of the St. Mary's Group of Hospitals, assisted by T. S. ZAHORSKY, B.S., M.D., Instructor in Pediatrics, St. Louis University School of Medicine, and Assistant Pediatrician to the St. Mary's Group of Hospitals. Pp. 430; 144 illustrations. Third edition. St. Louis: The C. V. Mosby Company, 1939. Price, $4.00.

ANOTHER edition of this useful little book has been published. While the Reviewer feels that a standard textbook is best for the beginning student, he concedes that there is a place for a book of this type. It can be recommended for anyone reviewing for an examination, for interns and for the busy practitioner who desires concrete and accurate information.

Numerous revisions have been made in order to bring certain phases of the subject up to date and these additions are presented succinctly and accurately.

One wishes that the author had not adopted so whole-heartedly the Robertson hypothesis of growth, which though attractive is highly debatable. An outline of the maturation of the skeleton according to Todd and of the maturation of motor development according to Gesell would be helpful. Since the active immunization of children with certain vaccines is still debatable it might be well to have the author state his preference in the form of recommendations rather than positive statements.

The illustrations are clear and distinctive, and there is a satisfactory working index. E. T., JR.

CLINIQUE ET PATHOLOGIE COMPAREE. Vénéréologie—Cancérologie—Dermatoses—Médécine Générale—Phyto-Pathologie. By LOUIS BORY, Chef de Clinique de la Faculte de Médécine de Paris a l'Hôpital Saint-Louis, Prix Duchenne de Boulogne, 1937. Pp. 239. Paris: Masson & Cie, 1939. Price, Fr. 50 (paper).

THIS book is a pioneer in extending comparative pathology into the plant field. It is certainly most unusual in systematic relating such diseases as lymphopathia venerea, for example, on the lower animals. "Syphiloid of the cat" is another example. The book should have a particular appeal to dermatologists for its paragraphs on scabies, demodex infestment, urticaria and prurigos, eczema, neurodermites, keratoses, "trophodermies," leprosy, subcutaneous nodular tuberculosis of cattle, syphilis, syphiloid of the cat and lymphopathia venerea. Having general appeal are the tumors, the leucoses, Johne's disease, leprosy, subcutaneous nodular tuberculosis of cattle, infectious icterus and epilepsy.

Veterinarians, among others, must be interested in the special chapter which deals with diseases of animals which may be transmitted to him, but the medical profession at large can profit also from it. Diseases mentioned are as follows: glanders, anthrax, rabies, scabies, ringworm, variola, tetanus, aphthous fever, erysipeloid, foot rot and tuberculosis.

The chapter on phytopathology is particularly in point at this time when viruses are being energetically studied in plant materials and leaves the feeling that a fuller presentation would be welcomed. The author devotes 16 pages first to the general organization of plants and then to the lesions met in them. The "tumors" induced by *Bacillus tumefaciens* are included.

This long list of subjects is crowded into 236 pages, without any illustrations, consequently the treatment is so sketchy that the content is only suggestive. The average reader who is now led into a new and strange field must feel that the content is inadequate to a satisfactory knowledge of the situation. Footnotes carry references to the literature, which are only partly adequate; nevertheless the book is decidedly thought provoking by indicating avenues of approach through animals and plants to problems in human disease. F. W.

OPHTHALMOLOGY. Vol. XX of Clio Medica. A Series of Primers on the History of Medicine. By BURTON CHANCE, M.D. Pp. 240; 6 illustrations. New York: Paul B. Hoeber, Inc., 1939. Price, $2.00.

THIS is an epitome of the development of ophthalmology from antiquity to the present time. The first chapters deal with the treatment of diseases of the eyes by the Babylonians, Egyptians, Chinese, Arabians, Greeks and Romans. Then follow sections on the development of the science of ophthalmology up to the 19th century. The remaining chapters take up specific ocular disorders and trace the development of our knowledge of these up to the present. It is delightfully written by an authority on the history of ophthalmology. F. A.

EYE, EAR, NOSE AND THROAT MANUAL FOR NURSES. By ROY H. PARKINSON, M.D., F.A.C.S., Head Oculist and Aurist to St. Joseph's Hospital, San Francisco, Calif. Pp. 243; 79 illustrations. Fourth edition. St. Louis: The C. V. Mosby Company, 1939. Price, $2.25.

A TEXTBOOK for nurses that contains excellent material, including diagrams covering the normal anatomy and physiology of the eye, ear, nose and throat and diseases of these structures, with very detailed instructions for the information of the nurse as to her responsibilities regarding treatment. The final chapter on "Public Health" describes the various

problems, in regard to these special conditions, that a nurse meets with in community nursing.

This book is the result of the author's belief that there is a need for it in the classroom and it has been written in the manner of classroom teaching, that is, in a conversational style, with considerable repetition and lack of clarity, as is natural in this form of writing. This is probably due to the fact that the author has written this book with his own immediate situation in mind; a better organization of the material would be a great improvement.

M. S.

THE SURGERY OF INJURY AND PLASTIC REPAIR. By SAMUEL FOMON, PH.D., M.D., Formerly Major in Medical Corps, U. S. Army. Pp. 1409; 925 illustrations. Baltimore: The Williams & Wilkins Company, 1939. Price, $15.00.

THIS extraordinary book is reminiscent of the older German textbooks in that it includes a full discussion of all possible fields related to the surgery of injury and tissue repair. The first chapter is devoted to a general discussion of the operating room set-up, asepsis, suture material and instruments, the incision, hemostasis, etc. There are separate chapters on the fluid, salt and acid-base balance, on shock, anesthesia, preoperative management and postoperative management. Each of these chapters has a comprehensive table of references, although some of these have not been critically selected. An introduction of this character is so unusual that it deserves comment.

The chapters on plastic surgery are extensive, well documented and include practically every operation of any importance. It is regrettable that with a text of such importance the illustrations, which of necessity had to be limited in size, could not have been done throughout with ink. The pencil drawings when reproduced in such small size are fuzzy in outline and frequently obscure. A suggestion, which applies to a number of books on plastic surgery as well as to this one, is that all illustrations be placed in the same direction, that is, in illustrations of operations on the palate the nose should be toward the top of the page. It is unnecessarily confusing to have to orientate oneself for various positions of the palate.

Of all the textbooks on injury and plastic repair that have appeared, this is undoubtedly the most extensive, but, of even more importance, it is unique in its advocacy of the knowledge and use of physiologic principles in the treatment of patients.

This is a valuable book for the specialist and also for the general practitioner who is called upon to repair superficial injuries. It can be highly recommended.

I. R.

MICROBIOLOGY AND PATHOLOGY. By CHARLES F. CARTER, B.S., M.D., Director, Carter's Clinical Laboratory, Dallas, Texas; Consulting Pathologist, St. Louis Southwestern Railway Hospital, Texarkana, Ark., and Mother Frances Hospital, Tyler, Texas, etc. Pp. 755; 165 illustrations and 25 colored plates. Second edition. St. Louis: The C. V. Mosby Company, 1939. Price, $3.25.

THE text is a brief but comprehensive survey of the fields of microbiology and pathology, in which bacteriology and related topics are emphasized. The section on microbiology has been thoroughly revised and broadened while the section on pathology remains essentially unchanged. The vast amount of material that is covered has necessitated the treatment of much of the subject matter in a definitive manner, while considerable space is devoted to the purely technical aspects of bacteriology and immunology. Generally, however, the subjects are presented in a simple, concise, factual

manner, and are supplemented with pertinent nursing suggestions, numerous illustrations, good references, an adequate glossary and index. Scientific nursing and its dependence upon an intimate understanding of the science of microbiology in relation to the nature of disease is emphasized throughout. A list of review questions covers each chapter and the section on microbiology is augmented by numerous simple and advanced laboratory exercises. The text is excellent from the standpoint of the graduate nurse or busy practitioner who wishes a sound, brief, easily readable and understandable reference to refresh and enlarge his basic understanding of disease; but is a bit too broad to be thoroughly grasped by the average student nurse in the time usually allotted for the study of these subjects. The noticeable variation in the density of the type will prove objectionable to the discerning reader. H. H.

THE INFANT AND CHILD IN HEALTH AND DISEASE. With Special Reference to Nursing Care. By JOHN ZAHORSKY, A.B., M.D., F.A.C.P., Professor of Pediatrics and Director of the Department of Pediatrics, St. Louis University School of Medicine, and Pediatrician-in-Chief to the St. Mary's Group of Hospitals, etc., and ELIZABETH NOYES, R.N., Supervisor and Instructor of Pediatrics, Children's Hospital, San Francisco. Pp. 496; 140 illustrations. Second edition. St. Louis: The C. V. Mosby Company, 1939. Price, $3.00.

A TEXTBOOK for nurses, which is not lacking in information needed in caring for children. The normal infant and child are appropriately considered first.

Then their diseases are thoroughly considered in a well-written account, with 481 illustrations and 7 color plates. The nursing procedures are particularly valuable. This book is one of the best textbooks for nurses. M. S.

THE PHYSIOLOGICAL BASIS OF MEDICAL PRACTICE. A University of Toronto Text in Applied Physiology. By CHARLES HERBERT BEST, M.A., M.D., D.Sc. (LOND.), F.R.S., F.R.C.P. (CANADA), Professor and Head of Department of Physiology; Associate Director of the Connaught Laboratories; Research Associate in the Banting-Best Department of Medical Research, University of Toronto, and NORMAN BURKE TAYLOR, M.D., F.R.S. (CANADA), F.R.C.S. (EDIN.), F.R.C.P. (CANADA), M.R.C.S. (ENG.), L.R.C.P. (LOND.), Professor of Physiology, University of Toronto. Pp. 1872; 497 illustrations and 2 colored plates. Second edition. Baltimore: The Williams & Wilkins Company, 1939. Price, $10.00.

FIRST published in 1937, and reprinted four times since then, this excellent book now appears in a new edition, thoroughly revised and including a large new section on the physiology of the special senses. Not only should students find it an ideal textbook, but physicians should welcome it as an excellent reference work to keep themselves abreast of modern concepts of physiology and the physiologic basis of scientific medical practice. R. K.

ELECTROCARDIOGRAPHIC PATTERNS. Their Diagnostic and Clinical Significance. By ARLIE R. BARNES, M.D., The Mayo Clinic, Rochester, Minn. Pp. 197; 94 illustrations. Springfield, Ill.: Charles C Thomas, 1940. Price, $5.00.

WRITTEN with the purpose of describing electrocardiographic patterns and pointing out their diagnostic importance in certain cardiac diseases, this monograph is not intended to furnish a complete discussion of electro-

cardiography. Thus nothing is said about the disturbances of cardiac rhythm.

The author is a seasoned veteran in clinical electrocardiography and for over 10 years has been one of the foremost investigators in this field. He is eminently qualified to prepare a monograph of this type. It is written in simple language and the thought is expressed clearly. The illustrations, an essential part of the monograph, are excellent. This sound, scholarly contribution should be in the library of everyone interested in electrocardiography. It will be particularly useful to the beginner and should be carefully studied by those who wish to qualify for the interpretation of electrocardiograms. It is a pleasure to recommend work of such high quality. C. W.

ASTHMA. By FRANK COKE, F.R.C.S. With the Collaboration of HARRY COKE, M.R.C.S., L.R.C.P., Honorary Physician, Charterhouse Rheumatism Clinic. Pp. 266; 19 illustrations. Second edition, fully revised and illustrated. Baltimore: The Williams & Wilkins Company, 1939. Price, $4.00.

SIXTEEN years having passed since the publication of its first edition, a drastic revision of the book was obviously called for. This has been only partly achieved, for there remains much old material that is not in harmony with current views of this rapidly growing subject. The same may be said of some of the theoretical concepts propounded, such as aspirin sensitivity or the sedimentation test as sure criteria for the classification of the major types of asthma. Literary references are at times poorly chosen (as when the leukopenic index is attributed to another instead of to Vaughan). From the clinical angle there is much of practical value, notably the mode of use of vaccine therapy. R. K.

INJURIES OF THE NERVOUS SYSTEM, Including Poisons. By OTTO MARBURG, M.D., Clinical Professor of Neurology, Columbia University; Research Neuropathologist, Montefiore Hospital, New York, etc., and MAX HEFLAND, M.D., Assistant Clinical Professor of Neurology and Psychiatry, Columbia University; Chief of Nerve Clinic, Post-Graduate Hospital, New York, etc. Pp. 213; 16 illustrations. New York: Veritas Press, 1939. Price, $3.00.

THIS comparatively short treatise comprises a wealth of information which is very important in view of the ever-mounting injuries resulting from traffic accidents. Marburg—formerly director of the Neurological Institute of the University of Vienna, now Clinical Professor of Neurology at Columbia University—reflects in almost every chapter the vast experience he had with injuries of the central and peripheral nervous systems during the World War. Even the seasoned neurologist will enjoy the comprehensive discussion of the symptomatology. In addition to the generally accepted characteristic signs of head and spinal cord injuries, one will find here a great number of the less known but very helpful methods of examination.

The practical viewpoint in diagnosis and treatment is stressed throughout. However, enough of the physiology and pathology is given to help the reader in the understanding of the symptoms and signs involved. Three photographs of macroscopic lesions and 13 well-selected photomicrographs from Helfand's investigations in vasomotor lesions in the brain following trauma are added. The general symptoms of head injuries and the special signs, diagnosis, pathology and treatment of concussion and contusion are discussed. The various forms of intracranial hemorrhages, of softening,

edema and cerebral atrophy, the fractures of the base of the skull and the injuries of the cranial nerves are described along with the neurologic sequelæ of concussion and contusion.

Five chapters deal with injuries of the spinal cord and the peripheral nerves, electric injuries, caisson disease and with trauma as the cause of organic nervous disease. The last chapter gives, in 10 pages, a concise résumé of the more common industrial intoxications such as lead, mercury, arsenic, thallium, manganese, benzol, benzene, carbon monoxide and carbon disulphide.

The importance and safeguards in evaluating pulse and blood pressure, the prognostic meaning of increase and decrease of body temperature, the value of pupillary disturbances as localizing signs, and the examination of the vasomotor system are discussed. The problems of traumatic necrosis and traumatic epilepsy are considered, and likewise, the percentages of partial and complete disability following the various kinds of injuries to the nervous system are considered at some length. The surgical treatment recommended for brain abscess, subdural hematoma, and traumatic epilepsy is at variance with the experience in this country. Some typographic mistakes and two errors in the labeling of the photomicrographs should be corrected in the next edition. The bibliography of over 300 references facilitates the orientation of the student.

The book is recommended as a short and valuable manual to internists, neurologists and surgeons, especially in the general hospital where a large number of head injuries are treated. F. L.

MANUAL FOR DIABETIC PATIENTS. By W. D. SANSUM, M.D., Chief of the Staff of The Sansum Clinic and Director of Metabolic Research of the Santa Barbara Cottage Hospital, ALFRED E. KOEHLER, PH.D., M.D., Member of the Staff of The Sansum Clinic and Member of the Metabolic Research Staff of the Santa Barbara Cottage Hospital, and RUTH BOWDEN, B.S., Dietitian of The Sansum Clinic, Santa Barbara, Calif. Pp. 227; illustrated. New York: The Macmillan Company, 1939. Price, $3.25.

IN a book of this kind, written for patients, perhaps the greatest temptation is to write into the text the author's own methods of treatment as well as information of a technical nature which should be found only in medical textbooks. In this excellent little book the authors have almost entirely avoided both these pitfalls. The language is clear and the explanations specific, and the material is restricted to generally accepted principles and facts which will help the patient to a better understanding of his condition and of how to aid his doctor in its care; in fact, it could not be amiss to say that for the general information which it contains many physicians could read this book with profit. One-third of the book is taken up with tables of food values, substitutions, recipes and a short glossary. One feels that this book may with safety be put into the hands of almost any patient with the assurance that from it he can learn all that is essential and useful for his care. R. R.

THE HOSPITAL CARE OF NEUROSURGICAL PATIENTS. By WALLACE B. HAMBY, M.D., F.A.C.S., Associate Professor of Neurology and Instructor in Surgery (Neurological Surgery), University of Buffalo School of Medicine, Buffalo. Pp. 118; 24 illustrations. Springfield, Ill.: Charles C Thomas, 1940. Price, $2.00.

THIS compact little book fills a definite niche in our textbooks for teaching nurses and hospital internes. It is the first, short, condensed account of the problems met with in the preoperative and postoperative care of neuro-

surgical cases. The author has covered the field concisely but at the same time omitted nothing of importance.

The chapters on anatomy are particularly good, as they give in brief form all that a nurse needs to know on the subject. The chapters on technique and treatment are concise but sufficiently detailed for use in the training school and instruction in the operating room.

The Reviewer can recommend this book very highly to those who teach surgery to nurses. They should be thankful that the author has taken the trouble to bring this information together, so that it will no longer be necessary to look it up from a number of sources or to bring out elaborate technique sheets covering this subject. F. G.

NEW BOOKS.

The Rise of Embryology. By ARTHUR WILLIAM MEYER, Professor of Anatomy, Emeritus, Stanford University. Pp. 367; 95 illustrations. Stanford University, Calif.: Stanford University Press, 1939. Price, $6.00.

Mineral Metabolism. By ALFRED T. SHOHL, M.D., Research Associate in Pediatrics, Harvard University (American Chemical Society Monograph Series). Pp. 384; 13 illustrations and 41 tables. New York: Reinhold Publishing Corporation, 1939. Price, $5.00.

Biological Products. By LOUIS GERSHENFELD, P.D., B.Sc., PH.M., Professor of Bacteriology and Hygiene, and Director of the Bacteriological and Clinical Chemistry Laboratories at the Philadelphia College of Pharmacy and Science, etc. Pp. 242; illustrated. New York: Romaine Pierson Publishers, Inc., 1939. Price, $4.00.

Lehrbuch der Augenheilkunde. By DR. ERNST FUCHS, Sechzehnte, vermehrte und verbesserte Auflage neu bearbeitet VON DR. ADALBERT FUCHS, a. o., Professor der Augenheilkunde an der Universität in Wien. Pp. 904; 362 text illustrations and 5 colored plates. Wien: Franz Deuticke, 1939. Price, Paper, M. 27; Bound, M. 30.

Medicolegal & Industrial Toxicology, Criminal Investigation, Occupational Diseases. By HENRY J. EILMANN, PH.D., Director of Physicians' Laboratory Service of Toledo, Ohio; Lecturer in Bacteriology and Histology, Mary Manse College of Toledo. Pp. 324. Philadelphia: The Blakiston Company, 1940. Price, $3.00.

Medical Care (A Symposium). Law and Contemporary Problems. Vol. VI, No. 4, Autumn, 1939 (A Quarterly Published by the Duke University School of Law, PROFESSOR DAVID F. CAVERS, Editor). Pp. 185. Durham, N. C.: Duke University Law School, 1939. Price, 75c.

Principles and Practice of Aviation Medicine. By HARRY G. ARMSTRONG, B. S., M.D., Captain, Medical Corps, United States Army; Director, The Aero Medical Research Laboratory, Air Corps Material Division. Pp. 496; 86 illustrations. Baltimore: The Williams & Wilkins Company, 1939. Price, $6.50.

Unto the Fourth Generation. GONORRHEA AND SYPHILIS. What the Layman Should Know. By IRVING SIMONS, B.S., M.D., Fellow of the American Urological Association; Fellow of the New York Academy of Medicine, etc. Pp. 243; illustrated (by M. EMANUEL, M.D.). New York: E. P. Dutton & Co., Inc., 1940. Price, $2.50.

Apparently every publisher feels that he must have a book in a popular line on his publishing list, whether or not there is really need for such a book. One would be inclined to class this volume as just another "pop" presentation of the patient's side of venereal disease, its control and treatment; however, it has one outstanding feature, *i. e.*, the space devoted to gonorrhea, the stepchild of the venereal disease control movement. The book is unlikely to reach more than a thin layer of the victims of venereal disease, whose reading contingent, is an insignificant part of the population strata involved in the problem. J. S.

Congenital Cleft Lip, Cleft Palate and Associated Nasal Deformities. By HAROLD STEARNS VAUGHAN, M.D., D.D.S., F.A.C.S., Professor of Clinical Surgery, New York Post-Graduate Medical School, Columbia University; Attending Surgeon, New York Post-Graduate Hospital, etc. Pp. 210; 259 illustrations. Philadelphia: Lea & Febiger, 1940. Price, $4.00.

Beyond the Clinical Frontiers. A Psychiatrist Views Crowd Behavior. By EDWARD A. STRECKER, A.M., Sc.D., LITT.D., M.D., Professor of Psychiatry in the Under-graduate and Graduate Schools of Medicine, University of Pennsylvania, and Consultant and Chief-of-Service, Institute of the Pennsylvania Hospital. Pp. 210. New York: W. W. Norton & Co., Inc., 1940. Price, $2.00.

Argyria. The Pharmacology of Silver. By WILLIAM R. HILL, M.D., Instructor in Dermatology and Syphilology, University of Pennsylvania, and DONALD M. PILLSBURY, M.A., M.D., Associate Professor of Dermatology and Syphilology, University of Pennsylvania. Pp. 172. Baltimore: The Williams & Wilkins Company, 1939. Price, $2.50.

The Inter-relationship of Mind and Body. (The Proceedings of the Association, New York, December 27th and 28th, 1938). Editorial Board: FOSTER KENNEDY, ANGUS M. FRANTZ, CLARENCE C. HARE. Pp. 381; 28 illustrations and 10 tables. Baltimore: The Williams & Wilkins Company, 1939. Price, $6.00.

The Physical and Mental Growth of Girls and Boys Age Six to Nineteen in Relation to Age at Maximum Growth. By FRANK K. SHUTTLEWORTH. (Vol. IV, Serial No. 22, No. 3.) *Evaluations of Adolescent Personality by Adolescents.* By CAROLINE MCCANN TRYON. (Vol. IV, Serial No. 23, No. 4.) (Monographs of the Society for Research in Child Development.) Pp: No. 3, 291; No. 4, 83. Illustrations: No. 3, 150 and 110 tables; No. 4, 12, and 15 tables. Washington, D. C.: Society for Research in Child Development, National Research Council, 1939. Price, No. 3, $2; No. 4, $1.

Fundus Atlas. By LOUIS BOTHMAN, B.S., M.D., F.A.C.S., Clinical Professor of Ophthalmology, The University of Chicago; Associate Ophthalmologist, St. Luke's Hospital, Chicago, and REUEL W. BENNETT, Photographer for the Division of Ophthalmology, The University of Chicago Clinics. Pp. 50; Fifty original stereoscopic photographs. Chicago: The Year Book Publishers, Inc., 1939. Price, $17.00.

Atlas of Surgical Operations. By ELLIOTT C. CUTLER, Moseley Professor of Surgery, Harvard University and Chief Surgeon of the Peter Bent Brigham Hospital; Formerly Professor of Surgery, Western Reserve University and Director of Surgery of the Lakeside Hospital, and ROBERT ZOLLINGER, Assistant Professor of Surgery, Harvard University and Senior Associate in Surgery at the Peter Bent Brigham Hospital. Pp. 181; 84 plates. New York: The Macmillan Company, 1939. Price, $8.00.

Medical Education in the United States, 1934–1939. Prepared for the Council on Medical Education and Hospitals of the American Medical Association. By HERMAN G. WEISKOTTEN, M.D., Dean, Syracuse University College of Medicine, ALPHONSE M. SCHWITALLA, S.J., PH.D., Dean, St. Louis University School of Medicine, WILLIAM D. CUTTER, M.D., Secretary, Council on Medical Education and Hospitals of the American Medical Association, HAMILTON H. ANDERSON, M.D., Member of the Staff, Council on Medical Education and Hospitals of the American Medical Association. Pp. 259. Chicago: American Medical Association, 1940. Price, $1.00.

Virus and Rickettsial Diseases with Especial Consideration of their Public Health Significance. A Symposium held at The Harvard School of Public Health, June 12–17, 1939. Pp. 907; illustrated. Cambridge, Mass.: Harvard University Press, 1940. Price, $6.50.

NEW EDITIONS.

An Introduction to Gastro-enterology. Being the Third Edition of The Mechanics of the Digestive Tract. By WALTER C. ALVAREZ, Professor of Medicine, University of Minnesota, The Mayo Foundation, and a Senior Consultant in the Division of Medicine, The Mayo Clinic, etc. Pp. 778; 186 illustrations. Third Edition. New York: Paul B. Hoeber, Inc., 1940. Price, $10.00.

A Textbook of Laboratory Diagnosis. With Clinical Applications for Practitioners and Students. By EDWIN E. OSGOOD, M.A., M.D., Associate Professor of Medicine and Head of the Division of Experimental Medicine, University of Oregon Medical School, and Member of the Staff of Multnomah County Hospital, etc. Pp. 676; 27 illustrations and 9 colored plates. Third Edition. Philadelphia: The Blakiston Company, 1940. Price, $6.00.

Recent Advances in Neurology. By W. RUSSELL BRAIN, D.M. (OXON.), F.R.C.P. (LOND.), Physician with charge of Out-patients to the London Hospital; Physician to the Maida Vale Hospital for Nervous Diseases; Neurologist to the Infants' Hospital, etc. Pp. 364; 24 illustrations. Fourth edition. Philadelphia: The Blakiston Company, 1940. Price, $5.00.

This edition is largely rewritten and discussion of the following subjects is new, or nearly so: Pathogenesis and Treatment of Headache; Functions of the Frontal Lobe; Electro-encephalography; Vitamin Deficiencies in Relation to Nervous Disorders; Diseases Due to Neurotrophic Viruses; Use of Prostigmin in Myasthenia Gravis; Chemistry of the Muscular Dystrophies; Muscular Disorders Associated with Thyroid Disease, and the Treatment of Meningitis with Sulphanilamide. There are many references and an index. N. Y.

Nomenclature and Criteria for Diagnosis of Diseases of the Heart. By The Criteria Committee of the New York Heart Association, ARTHUR C. DEGRAFF, M.D., CLARENCE E. DE LA CHAPELLE, M.D., CARY EGGLESTON, M.D., CHARLES E. KOSSMANN, M.D., ROBERT L. LEVY, M.D., JOHN B. SCHWEDEL, M.D., HAROLD E. B. PARDEE, M.D., Chairman. Pp. 282; 52 illustrations. Fourth edition. New York: New York Heart Association, a Division of New York Tuberculosis and Health Association, Inc., 1939. Price, $2.00.

Disorders of the Blood. Diagnosis, Pathology, Treatment and Technique. By LIONEL E. H. WHITBY, C.V.O., M.C., M.A., M.D. (CANTAB.), F.R.C.P. (LOND.), D.P.H., Assistant Pathologist, The Bland-Sutton Institute of Pathology, The Middlesex Hospital; Late Pathologist, The Children's Hospital, Hampstead, and C. J. C. BRITTON, M.D. (New Zealand), D.P.H., Assistant Pathologist, The Bland-Sutton Institute of Pathology, The Middlesex Hospital; Pathologist, The Samaritan Free Hospital for Women; Late Assistant Pathologist, Christ Church Hospital, New Zealand. Pp. 603; 61 illustrations with 12 plates (8 colored). Third edition. Philadelphia: The Blakiston Company, 1939. Price, $7.50.

It is not surprising that new editions of this excellent book are required every 2 years, especially if one realizes how rapidly hematology has been progressing in the past decade. One is more apt to get satisfactory and authoritative information on many subjects from this small book than from some of the larger works on the subject. "This edition contains important new matter concerning the hemolytic anemias, including more exact methods for the fragility test and for determining bilirubinæmia, views concerning the technique and value of sternal biopsy as judged by our own experience, as well as modern opinions about certain of the hæmorrhagic states."

PROGRESS
OF
MEDICAL SCIENCE

PATHOLOGY AND BACTERIOLOGY.

UNDER THE CHARGE OF
W. L. HOLMAN, M.D., C.M.,
PROFESSOR OF BACTERIOLOGY, UNIVERSITY OF TORONTO, TORONTO, CANADA,
AND
G. LYMAN DUFF, M.A., M.D., PH.D.,
STRATHCONA PROFESSOR OF PATHOLOGY, MC GILL UNIVERSITY, MONTREAL, CANADA.

STAPHYLOCOCCUS TOXIN; A RÉSUMÉ.

I. Introduction. It would seem appropriate at this time to review the problem of the production, preservation and the pathologic lesions produced by staphylococcus toxin. Recent observations indicate that this toxin and the toxoid and antitoxin prepared with it are not only of scientific interest but they are assuming clinical significance.

Both French and German investigators began the study of staphylococcus toxin following the observations of Billroth[6] and Koch[49] on this bacterium. Many publications appeared between 1874 and 1908. Relatively few papers appeared between 1908 and 1925.

Interest in the staphylococcus and its toxin was revived following the Brundenberg disaster which occurred in Australia in 1928.[16] At that time a group of 21 children were given diphtheria toxin-antitoxin and 12 died within the following 24 hours. A British Royal Commission investigated this disaster and found that the antigen was contaminated with *Staph. aureus*. The children died apparently from an acute intoxication, no doubt resulting from this staphylococcus. Abscesses formed at the site of inoculation in each of the 9 children who survived. A staphylococcus was isolated from these lesions similar to that present in the toxin-antitoxin used for the inoculum. The results of this disaster stimulated both clinical and experimental studies on the staphylococcus and its toxin particularly in England and in America.

Burnet[14a,b,c] in Australia, Dolman[24a] in Canada, and Parker[72–74,98] in the United States, made several important contributions pertaining to staphylococcus toxin between 1925 and 1932. In 1929, Burnet[14a] first prepared staphylococcus toxoid by treating staphylococcus toxin with formaldehyde. The experimental and clinical use of this staphylococcus antigen immediately attracted the attention of investigators. Dolman deserves credit for the early observations on the clinical use of this antigen. More recently, the French have made many clinical studies with staphylococcus toxoid and antitoxin.

The early workers on staphylococcus toxin thought that it contained

several different fractions. Those most frequently discussed were leukocidin, hemolysin, acute killing fraction, skin necrotizing fraction and nephrotoxin.

II. Production of Staphylococcus Toxin. The production of toxin by the staphylococcus is influenced especially by the medium and the duration of growth. Attempts to determine the best medium for the production of toxin have resulted in a considerable number of experimental studies. One of the earliest of these media was veal bouillon used by de Christmas[18] in 1888 to obtain the toxin which he injected into the anterior chamber of a rabbit's eye. It induced edema and suppuration. Van de Velde[93] grew staphylococci in blood, serum and bouillon to demonstrate the effect of the filtrate on leukocytes. He showed the presence of a substance which destroyed leukocytes.

Neisser and Wechsberg[62] in 1901 apparently were the first to give considerable attention to the conditions under which staphylococcus toxin is formed. Meat infusion broth was their basic medium. This has continued until the present to be basic in the preparation of staphylococcus toxin. Bigger[5] and his co-workers in 1927 were the first to digress from liquid media for the production of toxin. They grew the cocci on an agar surface and washed them off with saline to obtain the toxin. The medium used at present for the production of staphylococcus toxin is a modification. The proportions of ingredients are such now that a semisolid medium is usually formed.

McIlwain[55] has recently studied the effect of agar on the production of alpha hemolysin. His observations show that the production of toxin in a chemically defined medium is reduced by the calcium in the agar and that it is increased by the remainder of the agar molecule. The stimulating properties of agar are not due to its sulphuric ester grouping but to the polysaccharide residue. Agar in the medium used for the production of staphylococcus toxin may act by absorbing metabolic products deleterious to the production of the toxin.

Holt,[40] in 1936, studying the conditions under which staphylococcus toxin is formed, divided the nutritional elements in a beef-juice medium into large and small molecules by either saturation with ammonium sulphate to obtain the former or by dialysis to get the latter molecules. The yield of toxin in the "dialysate medium" and in the undiluted dialyzed medium was usually identical but the growth of bacteria was much greater on the latter.

Many substances have been added to the basic medium in an attempt to increase the production of toxin. Different preparations of peptone apparently have little influence on the amount of toxin that is formed. Walbum[96a,c] concluded from his studies that a 1% solution was the minimum in which staphylococci would grow and produce a lysin. An albumin, according to Walbum, is not an important factor for the growth of staphylococci and the formation of toxin. Glucose in a 1% concentration prevents the production of toxin.[96c] Parker[72] thinks that toxin only appears later when the medium contains a trace of glucose and that it probably does not appear until the sugar is destroyed. Certain salts such as calcium and sodium either partially inhibit or completely prevent the formation of staphylococcus toxin.[98] Salts of manganese, nickel, magnesium, and potassium when added to the

medium promote the formation of toxin. The rôle played by these salts is not understood. It would appear that some may act as "buffers."

Neisser and Wechsberg[62] and Walbum[96c] have considered the importance of "buffers" in the growth of staphylococci and the production of toxin. The latter found that the maximum amount of toxin was produced at pH 6. Other investigators, however, produce good toxin in a more alkaline medium. Hemolysins are present at a pH of 8–9. Burnet[14b] buffered his medium by placing it in a McIntosh and Fildes anerobic jar and replaced 10% of the air with carbon dioxide. Subsequent observations indicate that 20% carbon dioxide in 80% oxygen is a better buffer than 10% carbon dioxide alone.

The length of time required for the staphylococci to grow and produce the maximum amount of toxin is influenced by several factors. One of these is the temperature. Walbum[96c] found the best to be around 40° C. The maximum amount of toxin is formed after 2 to 3 days. Some investigators in producing toxin have grown their cultures for 9 to 13 days. More recent experiments would indicate that 48 hours is a sufficient incubation time at 37.5° C. for the production of toxin. There may be some deterioration of the toxin when the cultures are incubated for several days.

Staphylococcus toxin is separated from the medium by filtration. The filters more frequently used are the Berkefeld, Seitz and Chamberland. Burnet[14b] observed that filtration through a Seitz did not affect the hemolytic titer appreciably. Personally, I was unable to determine any difference in the amount of hemotoxin before and after filtering a large quantity of a culture of staphylococci grown in a semisolid medium through a Berkefeld V candle. Nélis[63] noted that the enterotoxic substance in staphylococcus cultures was retained by an L_2 filter but it did pass a Seitz.

The choice of a method for filtering staphylococcus toxin is influenced by the constituents in the medium. The cultures can be centrifuged to eliminate many of the large particles before they are filtered. When agar has been used in the medium it is much better to filter before the culture becomes chilled.

Many efforts have been made to purify and concentrate staphylococcus toxin. The results apparently have not been as encouraging with staphylococcus as they have been with tetanus and diphtheria toxin. Bigger[5] and his associates were able to dry the supernatant fluid obtained from washing the surface of agar slants by placing it in a petri dish and keeping it at 37° C. overnight. The residue was a yellow film with a rather rancid odor which could be reduced to a powder form. When water, equivalent to the amount of liquid evaporated, was added, a cloudy fluid formed which was as active in producing hemolysis as the original filtrate. The dry lysin was very stable. The toxin could be concentrated by this method. Burnet and Freeman[15] precipitated staphylococcus toxin with either alcohol or ether in the cold and adsorbed the precipitate on aluminum hydroxide. The toxin was released with either Rochelle salt or a weakly alkaline solution. Staphylococcus toxin can also be precipitated with acetic acid. A yield of 60 to 90% has been obtained by this method. The bulk of the nitrogenous constituents in the crude toxin were also removed. When the latter method

of purification is used there is an increase in the units of toxin per milligram of nitrogen. This experiment indicates that the toxin has no definite relation to the amount of nitrogen in any single preparation. Only a small part of the acid precipitate consists of toxin; metaproteins, nucleo proteins and other acid precipitated nitrogen compounds constitute the greater part of the precipitate.[15]

Holt[40] concentrated staphylococcus toxin as follows: the filtrate was saturated with ammonium sulphate and centrifuged. The supernatant liquid was discarded and the precipitate was redissolved in water. By this method 90 to 98% of the toxin was recovered. When concentrated and purified as above, the toxin is said to be more stable than the raw material. The antitoxin-binding and hemolytic powers remain constant for many months, indicating according to Holt that very little or no autotoxoiding action takes place.

Most investigators have not only been interested in determining the type of medium and the environmental conditions under which the staphylococcus produces the most toxin, but they have also been interested in determining the mechanism of the formation of this toxin. At present the chemical composition of staphylococcus toxin as well as that of other toxins is not known. Recent experiments have shown several interesting factors about the formation of this toxin and they indicate that additional information along this line may give pertinent data pertaining to the composition of staphylococcus toxin. Knight[48] found that the accessory substances necessary for the growth of staphylococci were either nicotinic acid and vitamin B, or closely related substances. Toxin has not been produced in significant amounts until recently in a synthetic medium. Gladstone[34] has published a paper on "The Production of Staphylococcal Alpha-Hemolysin in a Chemically Defined Medium." In this paper the different constituents in the medium are discussed as well as the environmental conditions under which staphylococcus toxin is formed. With the knowledge of the growth requirements of the staphylococcus, studies on synthetic media may reveal important facts about the formation and composition of this toxin.

Holt[40] has obtained growth and produced toxin in a medium made with the dialysate from nutrient broth. McClean[56] observed that agar added to broth enables the staphylococcus to produce a potent toxin. I have made similar observations on the use of agar in media and have also found that gauze cut into small pieces and added to veal infusion broth enhanced the production of toxin. Cellophane, according to McClean,[56] exerts some influence on broth which renders it suitable for the production of toxin. These observations made with agar, gauze and cellophane on the production of toxin suggest the possibility that adsorption may enter the process of toxin formation, but nothing specific as to the composition of toxin, however, has been determined.

Eaton[28] has stated very appropriately, I think, that a constituent of the medium need not necessarily act directly either by stimulating growth or increasing the toxigenicity of the culture. It may act either by removing an inhibitory factor, by effecting changes in pH, reducing conditions during growth, or by protecting the formed toxin from destruction. Traces of unknown impurities in an "essential" substance

may even stimulate the formation of toxin. There is evidence, according to Eaton, to support the idea that toxins are synthesized in the bacterial cells and then either liberated by diffusion or by disruption of the cells. Eaton, in discussing staphylococcus toxin, states that it is possible that the enterotoxic substance present in cultures of staphylococci is a metabolite and not a true toxin.

III. Preservation of Staphylococcus Toxin. Staphylococcus toxin, like other bacterial toxins, deteriorates spontaneously. The rate at which it loses its toxicity is much slower, however, than that of diphtheria. Oxidation and reduction may play a rôle in the spontaneous deterioration of staphylococcus toxin. Apparently no one has determined whether or not staphylococcus toxin is antigenic following this form of deterioration.

Bigger[5] and his co-workers found that dry staphylococcus lysin was very stable. A sample kept at cold-room temperature in a desiccator for 2 months had exactly the same titer as when it was fresh. Filtrates preserved in the ice-box also do not lose their potency as rapidly as when kept at room temperature. Jordan and his associates found that staphylococcus toxin retained the original strength for at least 3 to 4 weeks when kept cold.[44] All observations on the spontaneous deterioration of staphylococcus toxin, however, have been made over periods too short for the data to be of much significance.

Staphylococcus toxin deteriorates very rapidly when highly diluted with physiologic saline. It is of interest to note that the hemolysin and the skin necrotizing factors both are affected similarly by this diluent. Burnet[14c] observed that staphylococcus toxin deteriorates much less rapidly in infusion broth than in saline. This toxin also deteriorates at different rates when diluted with saline and infusion broth extracts of different rabbits' tissues.[81] Regardless of all the observations on staphylococcus toxin, little is known of the mechanism of deterioration.

Many observations have been made on the effect of heat on staphylococcus toxin. Frequently the data are very meager, yet, all the experiments show that it is destroyed by heat. The amount of heat required to completely inactivate staphylococcus toxin, according to Gengou,[33] is influenced by the pH of the filtrate. The differences in pH may account for the wide range of temperatures which have been recorded as being necessary for the complete inactivation of staphylococcus toxin. A detailed study of the effect of heat on the hemolytic and skin necrotizing factors in staphylococcus toxin has recently been reported by Rigdon.[79j] It was found that the rate of deterioration is influenced both by the length of time at which the toxin is heated and the temperature. Toxin heated at 45° C. for 2 hours loses approximately 9% of its hematoxin, while the same toxin heated at 54.03° C. loses over 99% of its toxicity during the same period. Toxin heated for 2 hours at 57° C. still shows a trace of the hemolysin. The nature of the change induced by heat is not clearly understood.

Staphylococcus toxin is detoxified through the action of certain chemicals and it may or may not be antigenic. Burnet[14a] was the first to completely detoxify staphylococcus toxin with formaldehyde. The binding power of this treated toxin toward antitoxin is retained up to

about 50% of the initial value and the flocculation titer is practically unaltered.[15] Staphylococcus toxoid is an effective antigen capable of provoking high titer antitoxic sera in rabbits. The concentration of formaldehyde, the pH of the reaction and the temperature all influence the rate of detoxification. The loss of primary toxicity according to Burnet follows an approximate monomolecular course, *i. e.*, when the logarithms of the hemolytic titers are plotted against time a curve is obtained which approaches a straight line. It would appear therefore, according to Burnet,[15] that formaldehyde acts directly on the toxic groups without affecting other parts of the toxin molecule which are concerned only with antigenicity and combining activity.

Strong acids and alkalies destroy staphylococcus toxin very quickly.[63] There is little data to indicate that toxin thus treated is either antigenic or that it retains its combining power for antitoxin. Benzoate, sodium tartrate and lactic acid, according to Nélis, produce only a minimum detoxifying action on staphylococcus toxin, while salicylic, benzoic and especially B. oxynaphtolic acids produce a much greater detoxification.

Staphylococcus toxin is detoxified by shaking it with either chloroform or ether. In this method an equal volume of ether is added to staphylococcus toxin. The mixture is placed in a sealed tube and mechanically shaken. After a period of 10 minutes the toxin contains only 32% of the original amount of hemotoxin. When similarly treated for 48 hours only 0.46% is demonstrable. Staphylococcus toxin thus treated with chloroform is a very poor antigen. Johlin[41] thinks that the process of detoxification of staphylococcus toxin with both chloroform and with ether is one of interfacial adsorption. The effects produced by this surface adsorption are undoubtedly that of the catalytic action of surface concentration and orientation.

Li[53] was able to remove completely the hemolytic, dermonecrotic and lethal properties of staphylococcus toxin by the combined action of methylene blue and light. The toxin thus treated was equal in antigenicity to formol-toxoid and alum precipitated formol-toxoid. This method of detoxification is especially interesting since it is considered to be one of oxidation.

IV. Relation Between the Staphylococci and the Production of Toxin. The relationship between the cultural characteristics of staphylococci and the production of toxin has been frequently studied since Ogston,[68] in 1882, first suggested a classification for this bacterium. It was recognized very early that staphylococci from pyogenic foci produce pigment. This gave rise to the separation of the organism into the aureus and albus groups.

Neisser and Wechsberg[62] were among the first to note a variation in the amount of toxin produced by certain strains of staphylococci. Those isolated from carbuncles and deep seated foci of inflammation in the human were usually toxin producing strains. Staphylococci isolated from animals may produce toxin. Minett[59] found that staphylococci isolated from suppurative processes in dogs may produce a toxin which is highly active against sheep red corpuscles but inactive against rabbit red blood cells. This and other observations indicate that the hemotoxin produced by different strains of staphylococci may be different.

The type of hemotoxin produced by staphylococci apparently is

influenced by the environmental conditions under which the organisms have been growing and also the medium in which the bacteria are grown for the production of toxin. These factors have not always been considered in the studies on the type of hemotoxin produced by different cultures of staphylococci. Some of the observations on Beta toxin have been made with organisms isolated from animal sources.[59]

There appears to be no relationship between the quantity of toxin produced by the staphylococcus and the virulence of the organism for man. Recent observations suggest, however, that there may be some relation between the amount of hemotoxin necessary to lyse rabbit red cells and the virulence of the organism for the rabbit. Timmerman[91] has recently called attention to the variation in the susceptibility of red cells from different rabbits to staphylococcus toxin. I have also observed such a variation in rabbits' red cells. The difference in the susceptibility of red cells from different animals for toxin and the variation in the susceptibility of different animals for staphylococci has made it difficult to correlate toxin production and pathogenicity. Furthermore, to add to the complexity of this problem Bigger[4] has noted that the amount of toxin produced by any staphylococcus is not always constant.

It is well known that the cultural characteristics of the staphylococcus are variable. At present it is questionable whether or not there is any relationship between the ability of an organism to produce toxin, its virulence and its pathogenicity. McBroom[54] states, however, that hemotoxin production in staphylococci is definitely correlated with the extent and the speed of reduction of methylene blue to the leukobase.

V. Staphylococcus Coagulase and Fibrinolysin. Staphylococcus-coagulase is more frequently encountered in broth cultures of staphylococci although it does occur sometimes in certain of the staphylococcal filtrates.[29a] The association of the toxin and the coagulase is interesting.

The phenomenon of the clotting of blood by staphylococci or by the products of these organisms was first mentioned by Delezenne in 1898.[21] Many observations have been made since that time on the clotting of plasma by staphylococci or their filtrates. Gratia[37] and Gross[38] obtained the coagulase free from the bacteria by filtering a broth culture of staphylococci. The substance is sometimes heat stable; usually it is heat labile.[29] Gross considers it antigenic while Sudhues and Schimrigh[90] and Fisher[29a] thinks that it is non-antigenic.

The action of this coagulase on plasma is similar to that of an enzyme.[19] It may or may not be present in filtrates containing staphylococcus toxin.[17,19] This coagulasing substance appears in broth cultures within the first 3 days of incubation.[29] It can be precipitated from the filtrate with alcohol and concentrated in saline.[29] Coagulase is a substance elaborated by the staphylococcus. It is most closely correlated with the pathogenicity of this organism for the rabbit, according to Cruickshank[19] and others.[17]

The phenomenon of fibrinolysis and the effects produced by the plasma-coagulating substances should be considered along with a discussion of broth cultures of staphylococci. Filtrates, however, may sometimes contain it.[29b] Aoi[2] found that fibrinolysin resists heat (100° C. for 1 hour), direct sunlight and many antiseptics. It occurs

with greatest regularity in 5 to 14-day-old cultures.[29b] It may be precipitated with either 75% alcohol or acetone and it is antigenic.[29b]

Madison[57] found that 77% of the 78 strains of staphylococci isolated from superficial human infections had no fibrinolytic function, whereas 90% of 30 strains from "internal" human lesions were fibrinolytic. Fisher[29b] says that the fibrinolytic property of the staphylococcus appears to be inseparable from the live organisms in 24 out of 26 cases studied. Apparently staphylococci elaborate this substance according to Fisher as they grow in the clot. Some of the best plasma-coagulating strains show no coagulating activity toward fibrinogen. Fisher thinks that this may mean that either there is some substance in plasma and not in fibrinogen which aids in coagulation or that staphylococci may dissolve the fibrinogen before they clot it.[29b]

Our present knowledge is meager about staphylocoagulase and the fibrinolytic properties of staphylococcus filtrates. It can be said, however, that these substances are primarily two different things and that both are different from staphylococcus toxin. Aoi[2] reports that three reactions of the staphylococcus, the hemolysin, the coagulase and the fibrinolysin are intimately connected with the pathogenicity as determined by skin tests on rabbits.

Neter[66] has recently studied the fibrinolytic, anticoagulating and plasma-clotting properties of staphylococci. He found that the fibrinolysin dissolves human as well as animal plasma clot. Furthermore, it can be specifically neutralized by staphylococcus antiserum. The relation of the fibrinolytic, anticoagulating and plasma-clotting properties to the toxin produced by the staphylococcus was not considered, however, by Neter.

VI. Pathologic Lesions. De Christmas,[18] in 1888, apparently was the first to observe the local effect of staphylococcus toxin on any tissue. Acute conjunctivitis developed following an injection of it into the anterior chamber of a rabbit's eye. Conjunctivitis[1] may even occur when the toxin is instilled into the eyes of both human beings and experimental animals. Allen[1] has recently shown that a keratoconjunctivitis may also occur following the local injection of staphylococcus toxin into the eye. The lesion produced by staphylococcus toxin, according to Allen, is similar to that so frequently found in clinical cases of staphylococcus conjunctivitis.

Staphylococcus toxin when injected subcutaneously produces local necrosis. Von Lingelsheim,[95] in 1900, apparently was the first to produce this lesion in the skin of the rabbit. Since that time it has been frequently described. Parker,[72] in 1924, gave the histologic changes in the rabbit's skin following the local injection of this toxin. Recently I have studied the lesions produced by staphylococcus toxin in the subcutaneous tissue of mice.[79o] They are characterized by necrosis of all the tissue in the area. It is interesting that there are very few leukocytes present during the first 3 hours following the injection of staphylococcus toxin. This may indicate that leukocytes either do not reach the zone or that these cells are destroyed immediately by the leukocidic action of the toxin. After 3 hours apparently all the toxin has either united with the tissue or has been removed by the lymph and blood.

Leukocytes have invaded the area of necrosis at this time and apparently they are very active in phagocytozing the débris.

A potent preparation of staphylococcus toxin usually produces injury when injected into any tissue. Recently the lesions produced by this toxin when injected through the patella into the knee joint of rabbits were described.[79e] The acute process is characterized by edema and hyperemia of the periarticular tissues accompanied by a small amount of bloody fluid in the joint cavity. This acute reaction slowly subsides and there develops a chronic reaction accompanied by a proliferation of fibrous tissue around the joint. The cartilage of the ends of the bones may become eroded.

Focal areas of necrosis in the colon is another lesion that follows the local injection of staphylococcus toxin in the dog.[79d] The wall of the colon is edematous, and hyperemic 24 hours after the local injection of staphylococcus toxin. The mucosa is necrotic and areas are sloughing at this time. The lumen of the glands in the mucosa are frequently filled with degenerated epithelial cells and débris. The epithelial cells lining these glands show all degrees of injury from cloudy swelling to coagulation necrosis during the first 24 to 36 hours. The edema decreases considerably by the second to the fourth day following the injection of the toxin. Ulcers are present in the mucosa at this time. The margin and base of these ulcers are irregular and the adjacent tissue is infiltrated with leukocytes. Regeneration of the mucosa apparently begins very early and after 9 days many of the ulcers are covered by new epithelium.

Necrosis occurs in the brain of the dog in the areas injected with staphylococcus toxin.[79l] A similar lesion is produced by the same quantity of infusion broth injected into the brain of the same animal. In view of the latter lesion it would seem more likely that the necrosis following the local injection of staphylococcus toxin is due to trauma rather than to the direct action of staphylococcus toxin on brain tissue. Collections of red blood cells infrequently occur around the small blood-vessels in the cerebral cortex following an intravenous injection of staphylococcus toxin in the dogs. These collections of red cells are similar to other lesions which occur in the dog following the intravenous injections of this toxin.[79a]

Pathologic lesions occur following the oral administration of staphylococcus toxin. The association of staphylococci with certain cases of food poisoning makes the significance of this route very important. Borthwick[9] has introduced staphylococcus toxin into the stomach of laboratory animals. The animals died at intervals varying from 2 minutes to 5 days. Intense congestion of the mucosa of the stomach and duodenum associated with small hemorrhages in the tissue and effusion of blood into the lumen, congestion and hemorrhages in various internal organs constituted the most important postmortem lesions.

Borthwick[9] discusses the effect of the gastric secretions on staphylococcus toxin. His experiments indicate that this toxin can be absorbed through the intestinal mucosa of the rabbit and guinea pig. The sensitivity of staphylococcus toxin to slightly acid and alkaline reactions is of special interest in considering the frequency of the occurrence of staphylococcus food poisoning in the human.

Lesions have been frequently observed in the different viscera following the intravenous injection of staphylococcus toxin.[79a,l] The production of these lesions have been attributed to specific fractions in the filtrate. Van de Velde,[93] in 1894, first observed that leukocytes were destroyed by staphylococcus toxin. He gave the name leukocidin to that fraction in the toxin which destroyed these cells. A leukopenia develops following multiple intravenous injections of staphylococcus toxin in the rabbit.[46] Young myeloid cells appear in the circulating blood of these animals provided they survive the acute effects of the toxin for 36 hours or longer. The femoral bone marrow is hyperplastic in these animals if they live for 3 days or longer.[79l] These new cells appear to be of the myeloid series. Rabbits given staphylococcus toxin intravenously and dying within 24 to 48 hours usually show only focal areas of necrosis in the bone marrow. From these observations it would seem that staphylococcus toxin may destroy leukocytes both in the bone marrow and in the circulating blood.

Neisser and Wechsberg[62] introduced a method to study the effect of toxin on white blood cells. It was based upon the fact that living leukocytes reduce solutions of methylene blue. The method is particularly adaptable for quantitative studies.

Staphylococcus toxin may effect the erythroblastic series of cells as well as the myeloid following an intravenous injection. This was first noticed by Denis and Van de Velde[22] in 1895 and Kraus and Clairmont[50] in 1900. Since that time the action of staphylococcus toxin on red blood cells has been frequently studied in an attempt to learn something of the mechanism of hemolysis.

Kraus and Pribram,[51] in 1906, observed that certain filtrates of broth cultures of staphylococci kill rabbits in 5 to 30 minutes following an intravenous injection. Similar results have been obtained by others following the injection of staphylococcus toxin. The lethal factor has been applied to that substance in the filtrate responsible for this acute death.

Neisser and Levaditi,[61] in 1900, described areas of necrosis in the cortex of rabbits' kidneys following the intravenous injection of staphylococcus toxin. Nephrotoxin is the term applied to the fraction in the staphylococcus filtrate responsible for the production of this renal lesion. Similar areas of necrosis have been described by others in the kidneys of rabbits, cats and dogs.[32,36,79m,94]

Neisser and Levaditi[61] thought that these cortical necroses resulted from the occlusion of blood-vessels in the kidney by emboli; furthermore that these emboli were formed by groups of leukocytes which had been destroyed through the action of the leukocidin present in the same toxin. Rigdon, Joyner and Ricketts,[82] in 1934, expressed the opinion that this renal lesion resulted primarily from the direct action of staphylococcus toxin on the cells in the kidney. Glynn[36] more recently has shown that the mitochondria in the tubular epithelial cells of rabbits show evidence of injury within 5 minutes following an intravenous injection of staphylococcus toxin.

Focal areas of hemorrhage and necrosis have been observed following the intravenous injection of staphylococcus toxin in the gastro-intestinal

tract, heart, pericardium, lungs, liver, adrenals, mesentery, spleen, diaphragm and bladder.[79a,l]

The many lesions produced by staphylococcus toxin apparently have led to diverse opinions on the mechanism of the action of this toxin. There are several well established terms that are used at present to denote specific fractions in the toxin. Many attempts have been made to prove that these fractions are either the same or different substances. Much of the work of the early investigators supports the theory that staphylococcus toxin is composed of multiple fractions. Recent experiments, however, indicate that the toxin may not be so complex.

Neisser and Wechsberg[62] considered the leukocidin as a substance different from the hemolysin. Their experiments showed, however, that some leukocidin was adsorbed on red blood cells along with the hemolysin. Furthermore, the hemolysin as well as the leukocidin decreased when a filtrate was put with leukocytes. Another factor which indicates that the hemolysins and leukocidins are not the same substance is that antibodies for the leukocidin are produced in an animal immunized with only a lysin containing filtrate.

Wright[100] examined the filtrates prepared by the method of Parish and Clark from cultures of staphylococci of pathologic and non-pathologic origin, and found that alpha hemolysin and leukocidin as determined by the Neisser and Wechsberg technique were identical. Panton and Valentine,[69] using human neutrophils, found a leukocidin different from alpha hemolysin. Proom[75] has studied the relationship of the Neisser and Wechsberg type of leukocidin to the Panton-Valentine type and to alpha hemolysin. It appears from the latter's observations that the method of preparation of the toxins is of fundamental importance. Leukocidin may be present in filtrates from young cultures of certain strains of staphylococci which may not produce high alpha hemolysin. Both the alpha hemolysin and the Panton-Valentine leukocidin destroy the respiratory powers of the rabbit's neutrophils.

Weld and Gunther[98] state that they were able to adsorb on red cell stroma the substances responsible for sudden death, the renal lesion, hemolysis and the leukocidin. Nine-tenths of the hemotoxin and leukocidin was removed by the stroma, leaving the necrotoxin undiminished in the filtrate. It would appear unwise to conclude too much from this experiment in view of the wide variation in the susceptibility of rabbits to staphylococcus toxin; and furthermore Weld used only 2 rabbits to test the stroma absorbed filtrate.

Flaum and Forssman,[31,32b] in one of their many studies on staphylococcus toxin, absorbed the hemolysin on sheep red blood cells. Recently I titrated the hemolysin in a single preparation of toxin, using a 1% suspension of rabbit and sheep red cells, and found that the latter cells were not lyzed by a low dilution of the toxin. After being in contact with sheep cells for 18 hours this concentration of toxin when placed with rabbit cells lyzed them approximately the same as the toxin that had not been in contact with sheep cells. The results of the latter experiments indicate that either the toxin used was not adsorbed on the sheep cells or that a specific hemolytic fraction for these cells was adsorbed but that it was in a concentration too small to hemolyze the sheep cell.

Evidence has been accumulating since 1921 to show that there may be two and possibly more hemolytic substances in staphylococcus toxin antigenically distinct from each other.[11,35,60,83] Walbum[96b] noticed a peculiar type of hemolysis which took place in the ice-box after a preliminary period of incubation at 37° C. when goat cells were used. Bigger[5] and his associates at first concluded that the lytic substance responsible for the destruction of sheep cells was different from the rabbit-erythrocytic hemolysin. Furthermore, these two fractions show little or no antigenic activity. Bigger[5] later suggested, however, that the difference in the lysis of sheep and human red cells may be due to several hemolysins present in the filtrate, although such lysis could be explained by the presence of only one hemolysin and other accessory substances that inhibit or aid the hemolysis. Glenny and Stevens[35] found two antigenically separate substances in a single staphylococcus filtrate. They called these hemolysins alpha and beta. The former lyzed rabbit and sheep cells at 37° C., the latter hemolyzed only sheep corpuscles when they were placed in the ice-box. Bryce and Rountree[11] confirmed the observation of Glenny and Stevens[35] and reported strains of staphylococci that produced only Beta hemotoxin.

The Alpha hemotoxin is most frequently encountered in staphylococcus filtrates and apparently it is the one from which the international standard unit of antitoxin was prepared. If subsequent investigations substantiate the work of Smith and Price[87] that strains of staphylococci pathogenic for man produce a significant amount of Beta hemotoxin then it may be necessary to change the present standard unit of antitoxin.

The demonstration of two or more hemolytic substances in a staphylococcus filtrate; the limited knowledge which we have at present with regard to the formation of toxin and the mechanism of lysis of red blood cells, all contribute to a greater confusion when the subject of one or more fractions in staphylococcus toxin is discussed. Burnet,[14a] Parish and Clark,[70] Nélis,[63] and others think that staphylococcus toxin contains only one substance that produces lesions in many different tissues. Nélis[63] found that the rate of destruction of the hemolysin by heat paralleled the rate of destruction of the other toxic properties. I have also found that the rate of destruction of the hemolytic fraction parallels the rate of destruction of the skin necrotizing factor.[79j] Burnet[15] observed that all the fractions attributed to staphylococcus toxin were destroyed at a parallel rate by formaldehyde. The skin necrotizing and the hemolytic factor are also destroyed at the same rate when this toxin is shaken with either chloroform or ether. Travassos[92] and others have found that staphylococcus antitoxin neutralizes all the different fractions in staphylococcus toxin.

Other investigators, among whom there are Dolman,[25a] Parish,[70] Panton and Valentine[69] and Forssman[31] think that some of the fractions in staphylococcus toxin may be the same substance but that there are more than one responsible for the diversified effects produced by this toxin. Parish and O'Meara[71] express the opinion that the intradermic value of toxin approximates to the hemolytic value when rabbits' red blood cells are used. Panton and Valentine[69] find that the hemolysin and the dermo-necrotic toxins are closely associated and that there is no relation, however, between these and the leukocidin.

A gastro-intestinal or enterotoxic fraction was attributed to the staphylococcus first by Barber[3] in 1914. This substance has been studied subsequently by Jordan and his associates,[42,43a,b,c] Dolman,[24c,e,26,27] and many others. Staphylococcus filtrates containing this enterotoxic substance may produce vomiting, gastro-enteritis, diarrhea, fever and collapse in the human when taken by mouth.[24c] Some investigators have obtained similar symptoms following the ingestion of the filtrate in experimental animals. The mechanism of the effects produced by this toxin is not known. The pathologic lesions accompanying staphylococcus food poisoning apparently have been studied only in a few cases.[7]

Several controversies have arisen over this enterotoxic substance. Woolpert and Dack,[99] and Dolman[27] and his associates report that it is distinct from the toxic substance frequently produced by the staphylococcus. They contend that it is antigenic and that it is present after heating filtrates for 30 minutes at 100° C. Eaton[28] states that this enterotoxic substance may be a metabolite and not a true toxin. Jordan and Burrows found it non-antigenic and unlike other well-defined toxins; it is soluble in organic solvents.

Davison and Dack[20] attribute the following properties to staphylococcus enterotoxin: "It is relatively heat stable, but prolonged boiling or autoclaving gradually decreases its potency. It is not extracted from filtrates with chloroform. The varying results indicate that some undetermined factor controlled alcoholic precipitation. However, the enterotoxin retains its potency after treatment with alcohol, while the lethal staphylococcus toxin is destroyed. It is salted out of solutions saturated with ammonium sulfate, but not from those half saturated."

It is of interest to note that Woolpert and Dack could demonstrate gastro-intestinal toxin in none of the staphylococcus filtrates without other toxic substances also being present. There seemed to be a rough correlation between the amount of food poisoning substance and the other toxic factors.[99]

All the tests suggested for determining the presence or absence of "food poisoning strains" of staphylococci apparently have proven unsatisfactory. The results of the cultural method described by Stone[88] does not parallel that of Dolman's kitten test.[27] In the latter, young kittens are given an intraperitoneal injection of the filtrate. If they vomit the filtrate contains the enterotoxic factor. I found that kittens and young puppies would vomit following the intraperitoneal injection of infusion broth as well as with staphylococcus toxin.[79k]

There is very little definite information on the mode of action of staphylococcus toxin. Animals given a small amount of a potent toxin intravenously may die in 2 to 5 minutes. At autopsy, no gross lesions are demonstrable. Glynn[36] has found degeneration of the mitochondria in the tubular epithelial cells of the kidney after this short interval. Hemorrhages and capillary dilatation are the characteristic pathologic lesions found when the toxin is given intravenously and a period of only a few hours elapses before death. Focal and diffuse areas of necrosis occur in many of the viscera if the animal survives 15 hours or longer following an intravenous injection of a potent toxic filtrate. In view of these pathologic lesions it would seem that staphylococcus toxin injures directly the cells. There is a variation in the suscepti-

bility of the different cells to this toxin as shown by the difference in the pathologic process in the different viscera. The sudden death which so frequently follows the intravenous injection of staphylococcus toxin may indicate that a large number of cells are severely injured and an insufficient time elapses for these cells to undergo the histologic changes that are recognized as those following a severe injury. Animals that survive the acute effects of staphylococcus toxin die apparently as the result of the action of the toxin on the parenchymatous tissue. Such a form of death is well illustrated by those animals that develop renal necrosis following an intravenous injection. They show a marked retention of non-protein nitrogen in the blood at the time of death.[79m]

VII. Physiologic Effects of Staphylococcus Toxin. Some of the physiologic effects produced by staphylococcus toxin have been studied and apparently they are closely associated with certain pathologic lesions previously described. Russ[84] and Kellaway, Burnet and Williams,[47] have observed an immediate drop in blood pressure following the intravenous injection of staphylococcus toxin. Russ thought that this effect was the result of an obstruction in the pulmonary circulation. Kellaway and his co-workers were of a similar opinion. They also thought that there was some pharmacologic substance in the medium which in addition affected the blood pressure. Infusion broth when injected intravenously into anesthetized dogs produced a drop in blood pressure similar to that which occurs when staphylococcus toxin is injected into the same animal. In this experiment I was unable to determine whether or not the drop in blood pressure was due to the staphylococcus toxin or to some other constituent in the filtrate.[79c] In view of the injury to endothelial cells by the toxin it is very likely that there would be a fall in blood pressure following the injection of staphylococcus toxin. Until this toxin is obtained in a chemically pure form it would appear difficult to determine the effect of it on the blood pressure.

Nélis and Bouchaert[64] observed a progressive decline in the blood pressure which was also accompanied by a preliminary slowing of the heart and was followed by the appearance of extrasystoles and finally culminating in ventricular fibrillation. Dingle[23] and his co-workers, in studying the effect of staphylococcus toxin on the rabbit's heart with electrocardiographic tracings observed evidence of severe myocardial damage accompanied by venous engorgement preceding collapse and death. They concluded from their experiments that death resulted from the direct action of staphylococcus toxin on the myocardium.

Nélis and Bonnet[65] have observed the effect of staphylococcus toxin on the central nervous system. The toxin was injected into the cranial cavity and into the spinal canal. The effect is characterized by muscular contractions, convulsive movements, respiratory and cardiac difficulties and death. The rapidity with which death occurs is influenced by the quantity and potency of the toxin. Nélis thinks that staphylococcus toxin affects the respiratory center and that this is the mechanism of its lethal action.

VIII. A Consideration of the Mode of Action of Staphylococcus Toxin. The mechanism of the effects produced by staphylococcus toxin is suggested by the pathologic lesions and the physiologic changes observed. The mitochondrial changes described above in the renal tubular epi-

thelium apparently are not the only early acute injuries produced by this toxin. It is very probable that almost every functioning cell in the body is more or less severely damaged by staphylococcus toxin following the intravenous injection of a large dose. The dilated capillaries throughout the body and the presence of small hemorrhages suggest that this toxin injures the capillary endothelium. Blood escapes from the vessels as a result of this capillary damage. Staphylococcus toxin may therefore be considered an endothelial poison.

The cellular lesions which follow the local injection of staphylococcus toxin resemble those which occur in some of the viscera following an intravenous injection of the same filtrate. Coagulation of the cytoplasm in many of the cells throughout the body suggests that staphylococcus toxin may unite very readily with certain tissues. As a result of this union the cells are either partially or completely destroyed.

Levine,[52] considering the mechanism of the effects of the hemolytic fractions in staphylococcus toxin, concludes from his experimental studies that the so-called "hot-cold lysis" is not a special case, due either to "abnormal toxins" or to an interfering substance. It may be only an expression of the same basic principle which controls adsorption in general.

The rate of union of toxin with red cells may be influenced by the composition of the toxin as well as that of the cell. Seiffert[85] has shown that staphylococcus toxin is not fixed by the hemoglobin but by certain substances in the red cell, probably a lipoid or combination of lipoids. The formation of some substance by this lipoid and toxin alters the permeability of the cells and allows the escape of hemoglobin.

There are many observations to show that staphylococcus antitoxin given before a lethal dose of staphylococci or staphylococcus toxin inhibits death. This apparently would support the idea that toxin unites with cells to produce its injurious effect.

A single toxin may injure many different types of tissue. The degree of injury may be influenced by the type of cell as well as the concentration of toxin in the cell. In view of the effects of staphylococcus toxin on different tissues and the lack of experimental data to disprove it, there appears to be sufficient evidence to assume that staphylococcus toxin has at least one substance that lyses red blood cells, necrotizes the skin, destroys leukocytes, produces necrosis in the kidney and causes acute death of animals.

IX. Immunity. Staphylococcus antitoxin has been recognized since Van de Velde,[93] in 1894, described it along with his observations on leukocidin. This antitoxin is frequently found normally in the serum of humans and experimental animals. Bryce and Burnet[10] have studied the antitoxic content of the serum from individuals from birth to the seventh decade. They found that natural immunity is present at birth, presumably the result of a passive transfer from the mother. This antitoxic immunity is lost during the first few weeks of postnatal life. It slowly develops again during the first year and reaches the adult level at approximately the sixth year and remains more or less constant until old age, at which time a slight fall occurs.

The antitoxic content of sera found in adults may have resulted from unrecognized antigenic stimuli. There is a variation in the titer from

different individuals. The antitoxic content of sera can usually be increased by a series of injections of staphylococcus toxin, toxoid or by prolonged infection with certain staphylococci.[8,13a–d,25b,30,97] Animals with an increase in the antitoxic titer exhibit an increased skin resistance to the necrotizing effect of staphylococcus toxin, according to Dolman and Kitchings.[25b] There is no definite parallelism, however, between the increase in the amount of circulating antitoxin and the degree of acquired skin resistance to toxin.

Natural antistaphylotoxic immunity occurs in the rat and in the wild rabbit similar to that in the human.[10] There is some evidence which indicates that this immunity in the rat may be actively acquired through a series of infections.

Rats born from mothers immunized either during gestation or a very short time before pregnancy have a sufficient number of antibodies after 30 days to survive a lethal dose of staphylococcus toxin given intraperitoneally. In the study of this immunity it has been impossible to determine whether or not the antibodies passed directly from the mother to the fetus through the placenta or if the antigen passes the placenta and the antibodies are produced by the fetus.[79h]

The method of increasing the antitoxic titer by a series of injections of staphylococcus toxoid has offered an apparently satisfactory way to treat many of the clinical cases of skin infections due to the staphylococcus.[24b,77] This form of therapy, however, has not given satisfactory results in the treatment of osteomyelitis.[8,12]

Another method of immunization was possible when experiments showed the antitoxic titer of serum could be increased by a series of injections of either staphylococcus toxin or toxoid. The antitoxin thus formed neutralizes staphylococcus toxin both *in vivo* and *in vitro*[24d,45,67,70,77,79h,89] Pröscher,[76] in 1903, stated that "the first stipulation which we request for an antistaphylococcus serum which would have therapeutic value for man is that such would protect experimental animals against staphylococcus infections. This postulate has, up to now, not been filled." At present there is considerable experimental data to show that staphylococcus antitoxin given to rabbits will protect them against a lethal dose of a broth culture of staphylococci. This antitoxin also protects rabbits against the toxin derived from several different strains of staphylococci.[67]

Recently, in studying the effect of staphylococcus antitoxin on the reaction produced by toxin and non-toxin producing strains of staphylococci, it was found that mice previously passively immunized showed a marked increase over the untreated controls in the number of neutrophils and the degree of phagocytosis of the bacteria.[79c] The lesions produced by the toxin were also inhibited in the immune mice. These experimental results confirm the opinion expressed in 1936 by Ramon and his associates.[78] They stated that the antitoxin protected the cells and tissues against the necrotizing action of staphylococcus poison and thus made a less favorable medium for the growth and multiplication of the organism.

Smith,[86b] in studying the relationship between circulating antitoxin and resistance to experimental infection with the staphylococcus, found that the resistance of rabbits to the intravenous injection of strain

Wood 46 is correlated with the alpha antitoxin content of the serum. Antitoxin, however, does not prevent the development of localized staphylococcus lesions when the organisms are given intravenously. Mercier's[58] observations indicate that staphylococcus antitoxin may prevent lesions produced by the local inoculation of staphylococci.

The inhibitory effect of staphylococcus antitoxin on staphylococcus toxin may be shown *in vitro*. Antitoxin is added to blood agar at the time the medium is poured into petri dishes. Staphylococcus toxin, when dropped on the surface of blood agar, causes a zone of hemolysis to form; however, this lysis does not occur when the medium contains specific amounts of the antitoxin. Furthermore, hemolytic strains of staphylococci, when grown on the surface of blood agar media containing antitoxin, shows either an absence or inhibition of the hemolytic zone about the colonies.[79n] Sulfapyridine added to blood agar media inhibits the rate of growth of the colonies; however, it does not have any effect on the toxin produced by the staphylococcus.[80] Experiments now in progress show that sulfapyridine when given intravenously to rabbits does not prevent death produced by an intravenous injection of staphylococcus toxin.

Smith[86a] has presented some of the problems that may be encountered in the testing of antigens for comparative potency. She has also called attention to the inhibitory action of glycerol on the necrosis produced by staphylococcus toxin in the skin of the rabbit and the guinea pig. The action of glycerol, sucrose and glucose in inhibiting the necrosis is one of partial destruction of the toxin, according to Smith.[86c] I have observed a similar inhibitory effect produced by hypertonic solutions of sodium chloride on rabbits' red blood cells and also upon the skin necrosis produced by this toxin. When staphylococcus toxin is added to red blood cells in hypertonic sodium chloride solutions the degree and rate of lysis is inhibited. Apparently some change occurs in the red blood cell to render it refractive to the hemolytic action of staphylococcus toxin. Sodium chloride, added directly to staphylococcus toxin in a quantity sufficient to make a 12% concentration, does not destroy the hemolytic action.[79g] Rabbits injected intradermally with hypertonic solutions of sodium chloride and the bleb subsequently injected with toxin fail to show necrosis at the point of injection, but skin necrosis does occur below this site. The inhibitory action of sodium chloride on the dermal necrosis may be the result of the action of the salt on the tissue cells. The change effected may inhibit the union of the toxin and the cells. Staphylococcus toxin apparently is not neutralized by sodium chloride in the tissue since necrosis frequently occurs inferior to the point of injection of the toxin in areas previously treated with a hypertonic concentration of sodium chloride.[79b]

The experimental results in the treatment of staphylococcus infection would indicate two approaches to clinical therapy: first, an attempt to neutralize the toxin and, second, to increase phagocytosis of the bacteria. The choice of therapy in any case must be influenced by whether or not the staphylococcus produces a toxin. If the organism produced no toxin, then any method by which the number of phagocytes can be increased apparently is indicated. If the bacterium produces a potent toxin then an increase in the antitoxic titer is of prime importance. It

neutralizes the toxin before further tissue damage occurs. In any staphylococcus infection, immunity will be aided if the organism can be rendered more susceptible to phagocytosis either by increasing the opsonin or the agglutinins. The experimental results in a study on passive antitoxic immunity indicate that an increase in the number of leukocytes and an increase of phagocytosis occurs when the animal is given staphylococcus antitoxin.

Holman's[39] statement made in 1935 is still significant. "The progress during the last few years has greatly increased the knowledge of the mode of infection and wide potential powers of the staphylococci, and there is hope that more scientific methods of treatment and prevention may develop if all the various aspects of the problem can be properly correlated and effectively evaluated."

X. Miscellaneous. The standard unit of staphylococcus antitoxin was accepted by the League of Nations, Bureau of Biological Standards in 1935. It contains 0.0009502 gm. of standard staphylococcus antitoxin and is based on the hemolytic, skin-necrotizing and lethal factors in the toxin. These units have been accepted by the National Institute of Health and are as follows:

The hemolytic unit of toxin (H. U.) is the smallest amount of toxin which causes complete hemolysis (4+) of 1 cc. of a 1% saline suspension of fresh washed rabbit red blood cells.

The hemolytic test dose (L. H.) is that quantity of staphylococcus toxin which is just neutralized by 1 unit of United States Standard Antitoxin as is evidenced by inhibition of hemolysis.

The dermonecrotic dose (R. D.) is the smallest amount of toxin which causes necrosis in an area 0.5 cm. in diameter when injected intradermally into the skin of a healthy albino rabbit.

The dermonecrotic test dose (L. R.) is that quantity of toxin which is just neutralized by 1 unit of United States Standard antitoxin, as manifested by inhibition of dermonecrosis.

The minimum lethal dose (M. L. D.) is the smallest amount of toxin which causes the death of a healthy white mouse weighing between 17 and 22 gm.

The lethal test dose (L+) is that quantity of toxin which is just neutralized by 1 unit of United States Standard staphylococcus antitoxin, as manifest in the survival of the test animal (white mice).

Since this article on Staphylococcus Toxin was accepted for publication, Dr. J. E. Blair has published an interesting review article on "The Pathogenic Staphylococci" (Bacteriol. Rev., **3**, 97, 1939).

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PREVENTIVE MEDICINE AND EPIDEMIOLOGY.

UNDER THE CHARGE OF

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EPIDEMIOLOGICAL ASPECTS OF MOTTLED ENAMEL.

MOTTLED enamel is a characteristic abnormality of the teeth, caused by the ingestion of excessive amounts of fluorides. It ranks well up among the commoner dental anomalies, both in the extent of its distribution through the world, and in the number of persons it affects. The disease is of unusual public health interest because prevention is the only known method for its control. Once established, the condition persists throughout life because treatment is limited to efforts at cosmetic improvement, and natural recovery does not take place.

The results that have been attained in the study of mottled enamel during the past few years, serve as an excellent illustration of what can be accomplished through application of epidemiologic methods to the study of a non-communicable disease. As a matter of fact, a number of specialists in the field of public health have found an interest in mottled enamel. To the nutritionist the disease is unusual in that it is caused by an excess of the causative agent, rather than the deficiency with which he is so frequently concerned. The water-works engineer

encounters a disturbing situation, in that his traditional and ever present source of safe water, the deep well, too often gives rise to this disease through an excess of fluorides. To the dentist, the condition is unique in that it is the only well established anomaly which is sharply limited to endemic zones or geographic regions. The chemist has found an incompletely answered problem in attempting to develop simple, accurate methods for the quantitative determination of fluorine in food substances, and for the economical removal of fluorides from potable water.

Relation of Mottled Enamel to Other Forms of Poisoning by Fluorine. Distinguished from the several forms of chronic fluorosis—which include mottled enamel—are the occasional instances of acute poisoning. The most obvious lesions come from corrosive action on the mucous membranes, and on the skin. The clinical manifestations are in direct contrast with those of chronic fluorine intoxication, where systemic effects are absent unless the dosage is relatively large and long continued; and where the changes that follow are the result of selective localization of the chemical in tissues.

Roholm[47] summarized the literature of acute fluorine poisoning from 1873 to 1935, recording 60 fatal and 52 non-fatal cases. The most common sources were insecticides, rat poisons, and disinfectants. The clinical manifestations were those of acute gastro-enteritis, convulsions and paresis, with terminal vasomotor collapse. From animal experiments[49] and from observation of humans[48] a single dose of fluoride below the level at which toxic symptoms develop, has been shown to cause changes in the calcifying tooth characteristic of mottled enamel, although limited of course to a relatively small part.

Other pathologic and physiologic changes besides mottled enamel are known to result from chronic fluorosis. A characteristic sclerotic affection of the bones, which sometimes extends to adjacent structures, occurs in workers engaged in the cryolite industry[39,47] through exposure to dust containing fluorides. Bishop[8] reported similar bony changes in an industrial worker exposed for 18 years to rock phosphate dust. Shortt *et al.*[53] reported 10 adult patients with advanced fluorosis, where symptoms first appeared at about 30 years of age. Smith[56] was unable to find any bony changes in children with marked mottled enamel and felt that the teeth were much more sensitive to fluorine than the bony skeleton.

Rather recently interest has been given to a possible interrelationship between the metabolism of fluorine and thyroid activity.[26] Apparently fluorine can be stored[43b] in the thyroid in relatively high concentrations, although most of the evidence points against any direct relation between fluoride ingestion and actual enlargement.[26]

Despite the general assumption that defective teeth predispose to dental caries, McKay[36c,36d] long ago stated, that although representing the most poorly constructed enamel of which there is any record in dentistry, the teeth of mottled enamel show no greater liability to dental caries than do normally calcified teeth. Epidemiologic contributions of a number of workers[2,32] from several countries[4,25] have strongly indicated that the ingestion of fluoride bearing waters is associated with a decrease in the incidence of dental caries. That the active

principle is fluorine, has not been satisfactorily established. Other evidence,[38] while confirming the phenomenon, suggests that no greater importance can be attached to the fluoride content of drinking water than to its total hardness. It is possible that a wholly independent constituent is the answer. If fluorides do act as a protective factor in dental caries, that action is independent of the presence of mottled enamel, as indicated by the field studies thus far made in America.[14c,19] These investigations have been limited to children less than 16 years old, and have been principally concerned with regions where the usual concentration of fluoride in water is relatively low, although sufficient to cause mottled enamel. This associated interest in studies of mottled enamel is now attracting much interest.

The effects that follow ingestion of fluorine depend upon the dose, the mode of entry into the body, the duration of exposure, the type of fluorine compounds, the age of the individual, the composition of the diet and other less well understood factors. Until more is learned about these variables, and until the conditions that govern the secondary manifestations of chronic fluorosis can be determined, an understanding of mottled enamel would seem to rest on the premise that the calcifying tooth is more sensitive to the action of fluorine than are other tissues of the body, and that the milder forms of mottled enamel occur without other manifestations of chronic fluorosis.

Definition of Mottled Enamel. This hypoplastic abnormality of the teeth results from taking excessive amounts of compounds of fluorine into the body during that period of childhood when calcification of the enamel is under way. Under such circumstances, both dentin and the enamel of teeth are imperfectly formed.[7] When the tooth erupts, the enamel is abnormally opaque and chalky white, in contrast to the pearly, translucent appearance of normal enamel. White blotchy areas are usually interspersed with the areas of more or less normal enamel, a situation which has led to the very descriptive term of mottling.[9] Because this dental condition is the result of fluorine intoxication, usually over a long period of time, and because of its occurrence in localized geographical regions, the name "chronic endemic dental fluorosis"[14d] has recently been introduced.

This dental defect was first called *Denti di Chiaie*, having been recognized in Italy well in advance of 1901.[23] In Argentina it is known as *dientes veteados*. *Darmous*, a dental and mandibular disease among herbivora and man in certain parts of North Africa; and *gaddur*, a dental and bone disease among herbivora in Iceland occurring after volcanic eruptions, are undoubtedly more extreme forms of chronic fluorine intoxication, of which mottled enamel is only a part of the clinical reaction.

Since many health officers and practicing physicians have not had opportunity to become as familiar with mottled enamel as have dentists, consideration is next given to its clinical nature. There follows an account of the various etiologic factors. Principal emphasis is given to epidemiologic studies in the field, which have better defined the problem and as so frequently happens, have formed the basis for improved administrative practice for its control.

Clinical Recognition. When exposure to fluorides has been essentially continuous since birth, the condition of mottled enamel becomes clinically evident as soon as the permanent teeth have erupted. The cause lies in the action of fluorine at the time the enamel is in the process of calcification and hence takes place in the pre-eruptive stage of tooth development. Affected teeth are ill formed, rather brittle, dull and chalky white.

A definite relation exists between the amount of fluoride ingested, the fluorine content of the tooth, and the extent of clinical damage. When exposure has been great, the affected enamel tends soon after eruption to become pitted or even corroded. As a rule the pits and corroded areas are more pronounced on the labial surfaces and may be completely absent lingually. The pitting and corrosion are the macroscopic reflection of fracture of smaller or larger groups of enamel rods. When the exposure to fluorine has been both severe and continuous the tooth appears uniformly chalky instead of mottled.

A characteristic discoloration of the teeth is an added feature of more severe intoxications. This may range from light yellow to brown and even to black, and is commonly referred to as brown stain. It is never present when the tooth erupts and only appears in enamel already mottled. In contrast to other types of dental staining, it is located within the enamel structure, rather than on the surface. A most peculiar feature is the regularity with which it is confined to the outer surfaces of the upper front teeth,[36f] the lower incisors being spared. The disfigurement may be extreme.

Under exceptional circumstances, eruption of the teeth may be delayed, they may then be abnormally small, and may erupt in abnormal positions with resulting malocclusion.[28]

Because the permanent teeth calcify at different times and over a period of many years, and because calcification of the crown of any single tooth requires from 4 to 6 years, it is evident that with varying degrees and periods of exposure to fluorides, the extent and degree of mottling may vary, not only in different teeth but in parts of the same tooth. The ultimate effect from chronic fluorosis can only be determined years after the damage has been done. On the other hand, although ingestion of fluorides after the crown of the tooth has formed will sometimes result in a deposition of fluorine, especially in the pulp and dentin, it will not cause the changes in tooth structure characteristic of mottled enamel.

An accepted scheme for grading degrees of clinical reaction is essential for epidemiologic studies, and is often useful in clinical and public health practice. It must take into account the numbers of teeth affected, clinical severity as indicated by pitting and corrosion, and the presence and intensity of the stain. The simple but inclusive arrangement of Dean[14b] and his co-workers[17] is followed to good advantage in community surveys. Details are to be found in the original papers. The excellent illustrations of Dean, McKay and Elvove[20] and of Roholm[47] aid materially in the interpretation of the classification.

Experimental Production of Mottled Enamel. The first significant contribution toward determining the direct cause of mottled enamel was that by McCollum, Simmonds, Becker, and Bunting in 1925.[35]

Knowing that normal teeth frequently contain fluorine, they proceeded to determine experimentally the effect on tooth development of an excess of this element. When sodium fluoride was added to a normal diet of rats, in amounts but little more than many foods contain, marked abnormalities of the teeth constantly occurred. Although the lesions were similar to those of mottled enamel in man, described by Black and McKay[9] 9 years previously, no one associated the two conditions. Bergara[6] later described a symmetrical dark stain in the teeth of white rats, occurring 4 months after first receiving a diet containing sodium fluoride. Tolle and Maynard[60] confirmed the work of previous investigators, by feeding rats sodium fluoride. Continuing their experiments they used rock phosphate and triple superphosphate—both of which contain fluorine—in place of sodium fluoride. The changes that followed were the same. Chaneles[11] reported on the histopathology of the teeth of animals fed sodium fluoride.

These studies established that a characteristic abnormality of teeth could be produced by ingestion of fluorides. A possible connection with mottled enamel of man was not considered. Proof that the experimental and the naturally occurring disease were of similar nature and uniform cause, came from epidemiologic investigations in the field.

Epidemiological Contributions to Etiology. The first observations antedate experimental production of the disease by many years. Kühns,[30] in 1888, described anomalies of the teeth that are identifiable as mottled enamel. A family had returned to Germany from the town of Durango in Mexico. Those members of the family who had been born in Mexico had irremovable black spots in their teeth. He felt that the color of the teeth was possibly due to the presence of oxides of manganese resulting from the action of the sun on manganese salts deposited in the teeth from drinking water. Eager,[23] an officer of the United States Public Health Service, stationed in Naples, Italy, published in 1901 an account of his observations on defective enamel of the teeth among Italian emigrants, particularly those coming from Pozzuoli, a community near Naples. It was a matter of common belief, locally, that the condition was in some way concerned with the water supply and with volcanic emanations.

That the disease existed in America, first became generally known in 1902,[37] when practicing dentists in Colorado Springs undertook a systematic investigation. Beginning in 1906, Black[9] carried on extensive and fundamental studies of the disease over a period of 10 years, much of the work being in collaboration with McKay. A series of papers,[9,37] published in 1916, gave the name of mottled enamel to the condition and accurately described the endemicity and time factors involved. They presented supportive evidence to show that it was caused by certain water supplies. Subsequently McKay[36a] extended these observations to show close association with artesian waters. While thus relating the cause of the disease to common water supplies, they had no idea of the specific factor involved.

In 1925 McKay[36b] made a survey of Oakley, Idaho, in the course of which he found that the children of the town who used the municipal water supply regularly developed mottled enamel. Children from several families who used a nearby spring water were not affected. On

his recommendation, the latter supply was substituted as the source of drinking water for the town; this being the first instance of a community changing its water supply for the sole purpose of preventing mottled enamel.

A survey of Bauxite, Arkansas, made by Kempf and McKay[29] in February, 1928, might be termed the classical field study of this condition. Bauxite was a town established in 1901 to provide homes for the employees of a mining company. The original domestic water supply came from shallow surface wells and a few springs. As the population increased, a larger supply was required, and in 1909 a well was drilled to the depth of 255 feet, later augmented by two other deep wells. Most of the shallow wells were gradually filled in, and for the community proper the deep wells were the principal sources of supply. The 1928 survey included complete histories and dental examinations of 458 school children. From these data it was learned that no case of enamel defect antedated the introduction of the deep-well water. The oldest individual affected was born about the time that it was introduced. All persons in the community, who had used the deep-well water during any considerable period of enamel formation, exhibited the defect. Certain individuals, although residents of the community and attending school there, lived beyond the distribution of the deep-well water and depended upon the original shallow wells. They had normal enamel. Other environmental factors, and the mode of life and dietary habits of the people, were much the same in the village and outside the village. There was no differentiation by economic class. The use of water from the central deep wells during the time of enamel formation, seemed to be the single common factor. Persons intermittently exposed had mottling only of teeth in the process of enamel formation at the time of exposure. Other teeth were essentially normal. The extraction of a temporary molar from the mouth of a native Bauxite child showed the underlying bicuspid to be typically mottled, prior to exposure to mouth conditions. As a result of this survey the deep wells were abandoned. The new water supply was drawn from the Saline River, a source also used by the neighboring town of Benton where children showed no evidence of mottled enamel. Ten years after this change was made, a second study of Bauxite by Dean, McKay and Elvove[20] showed that mottled enamel among children of the community had ceased then just as suddenly as it had started when the deep-well supply was introduced.

Two separate lines of investigation had led to the situation in 1930 where a striking abnormality of teeth had been produced experimentally in the rat by feeding fluorides; and where a well established dental anomaly of humans had been related to water supplies. The two observations remained uncorrelated.

The many epidemiologic investigations had naturally led to detailed analyses of water supplies associated with mottled enamel, but examination for fluorides had been omitted. Influenced by the qualitative demonstration of fluorides in deep-well waters from Bauxite, Arkansas—these tests were made by Petry, but never reported—Churchill[12] extended the observation to waters from Oakley, Idaho, Colorado Springs, Col., and other towns where mottled enamel was known to be present. He found fluoride in all samples examined. He noted also that waters

containing appreciable amounts of fluoride tended to occur in certain geographic regions, but that mottled enamel was not present in all cities consuming fluoride bearing waters. His methods of analysis lacked sufficient quantitative accuracy to permit conclusion that the cause of mottled enamel was due to the ingestion of fluorides, but he suggested further investigations with this in mind. The work of Smith, Lantz, and Smith[58] definitely established the point in 1931.

Mottled enamel was prevalent in St. David, Arizona. From observations in that locality, Smith and her co-workers[58] first showed that the water supply contained from 3.8 to 7.1 parts per million of fluorine. They next proved experimentally that the lesions associated with chronic fluorosis in the white rat were identical with those of mottled enamel in man. Similar dental defects were produced in rats by feeding them sodium fluoride, and by giving them concentrated St. David water. A quantitative relation was found to exist between the amount of fluoride ingested and the presence of lesions. The amount required for development of the experimental disease in rats was greater than that which produced mottled enamel in humans. The explanation lies in the difference in rate of growth of the teeth, and also explains why animals are usually not involved in regions where humans regularly develop mottled enamel. These observations were promptly substantiated by Velu[61b] with proof that a similar ailment in North Africa, called darmous, was also chronic fluorosis.

Sebrell, Dean, Elvove, and Breaux[52] investigated the water of Conway, South Carolina. It contained 6 parts per million of fluorine, and had produced typical mottled enamel in humans. When concentrated by methods similar to those of Smith, Lantz, and Smith,[58] and fed to rats, it produced typical dental defects. They then made a synthetic drinking water, comparable to the concentrated natural Conway water and containing all of the ions present in amounts greater than one-half part per million, with the single exception of fluorine. This water produced no noticeable abnormality in the teeth of white rats. About the same time, McKay[36e] made a second survey of Oakley, Idaho, to determine the effect of changing from a water supply that contained fluorides to one that did not. Further development of mottled enamel had not occurred.

Pathogenesis. The gross and microscopic pathology of mottled enamel has been well described, but the manner in which fluorine causes these changes is still controversial. Fluorine is usually absorbed from the gastro-intestinal tract, very uncommonly from the lungs.[47] Surprisingly little is known of the form in which it is absorbed, circulated, stored, or excreted. As a general principle, fluorine is rather efficiently absorbed and but slowly excreted. Furthermore, it would seem that fluorine in low concentrations may have a stimulating effect, and in high concentrations be inhibitive to normal function of the invaded tissue. The concentrations giving rise to these paradoxical effects likewise vary with different animals, and for different tissues of the same animal. Some evidence has been presented to show that administration of relatively large amounts of fluoride causes a negative calcium balance,[34,47] inhibits certain enzyme systems,[21] affects the parathyroid glands,[13,46] and exhibits a special relation to vitamin C metabolism.[41,42,44]

Evidences of abnormal calcium-phosphorus metabolism and the other effects mentioned, have been demonstrated, however, only at intake levels much higher than is necessary to cause mottled enamel.

Most theories proposed in explanation of the mode of action of fluorine have been based on the assumption that fluorine in some way causes its adverse effects by direct toxic action on the enamel forming tissue. A close correlation exists, however, between the fluorine content of enamel and the severity of mottling,[3,10,33] regardless of the particular tooth, or the animal under consideration. Although all of the manifestations of fluorine storage and fluorine intoxication cannot be explained on a pure physico-chemical basis, there is a striking similarity between the behavior of fluorine in nature and its behavior in animals. In nature it is commonly found in combination with calcium (fluorspar), and with tricalcium phosphate (natural rock phosphate). Neither compound is very soluble in water, but natural rock phosphate is much less so than calcium fluoride. Several groups of workers[1,5,24b,31] have demonstrated that calcium fluoride in water is effectively and rapidly removed—to a certain minimal level—by tricalcium phosphate. In fact, this compound is one of the best agents for removing fluorides from potable waters. Since calcium phosphate, together with calcium carbonate, is the essential component of enamel and of bone, it is not surprising that Smith and Smith[54] obtained good results by using ground bone for the same purpose. In animals and man, the best evidence indicates[46] that fluorine occurs in the teeth and in the bones as a fluorapatite-like compound not greatly dissimilar to that found in nature as natural rock phosphate. There is considerable evidence, then, of a physico-chemical affinity between fluorine—especially calcium fluoride—and calcium phosphate, and that this affinity manifests itself both in nature and in the living organism.

A clearer idea of the mechanism by which the condition of mottled enamel is established in man, can perhaps be obtained by considering the kinds of change possible in bone generally, when exposed to fluorides. A more or less constant metabolic process takes place in bone,[51] with an interchange of components. Thus, sufficient amounts of the tricalcium phosphate component are free to act at any time, with formation of a fluorapatite-like substance. With very small amounts of fluorine entering the circulation, the changes that follow can be conceived as being of such slight moment that no demonstrable lesions become evident, and the process is comparable to simple storage. With larger amounts, the substitution would be sufficient to produce excessive amounts of abnormal fluorine-containing bone, an increase in the size of the bone and a pathologic condition called by Roholm[47] osteosclerosis. At very high levels of fluorine absorption,[47] as in gaddur, there is a toxic inhibitory effect on bone itself, associated with manifest generalized toxic symptoms. Presumably low grade metabolic changes in the bony substance would continue even when appreciable amounts of fluorapatite were present. If exposure to fluorides ceased, or continued only in slight degree, normal rather than fluorine-containing bone would tend to be reformed. This is consistent with observed recession of abnormal bony changes when exposure to fluorides ended.

The enamel of teeth, however, is not subject to this constant meta-

bolic interchange of chemical constituents. Calcium phosphate and other elements which go to make up the enamel are free to combine with such unusual substances as fluorine, only when the enamel is being formed. Thereafter, enamel behaves like a dead tissue, whether it is normal or abnormal.

It does not seem physiologically sound furthermore, to postulate that larger dosage is required to injure a rapidly growing tissue, than one growing slowly—the explanation commonly given for the larger amounts of fluorine needed to produce the disease in the rat compared with man. Explanation of the phenomenon would seem to rest equally well on a purely physical basis. With more newly formed enamel in a given time, more fluorine would be required to produce an equal effect. This is offered as a more reasonable explanation than the theoretically enhanced toxic action on young growing tissues.

Sources of Fluorine in Nature. Fluorine has been estimated[64] as thirteenth in order of importance among the elements composing the earth's crust, thus placing it ahead of any of the other halogens and far ahead of such toxic elements as lead, arsenic and mercury. As industry has advanced, the use of fluorine compounds has progressively increased. Industrial development in turn was one of the important factors leading to an increased urban population, the greater need for community water supplies, and consequently the greater use of deep-well waters. In the United States the development of the west resulted in a shift of population to regions now known to be fluoride areas. In all of these ways, the potential exposure to fluorine compounds increased.

The recognized importance of fluoride bearing waters in chronic endemic dental fluorosis, and the relative ease with which the element can be determined in water, are the principal reasons for the major attention this source of fluorine has received. The widespread distribution of compounds of fluorine in nature requires consideration of other sources—in food, in association with industry, from medicinal substances, and in some localities, contact with volcanic dusts.

A number of reasons exist why more attention has not been given to these other potential sources of fluorine. The causal relationship to mottled enamel was only established in 1931. The possibility of other health aspects has been recognized still more recently. Accurate quantitative methods had to be perfected for examining the diverse materials concerned. That primary attention was given to endemic areas where whole populations were affected, is also understandable. The situation is in many ways comparable to the evolution of our attitude toward typhoid fever. To date we have been attracted by epidemics, as was Budd in his early studies of that disease. As epidemics become less frequent, attention must shift to sporadic cases, to mild and low grade affections, and perhaps even to the prototype of subclinical infection in typhoid. This will require better understanding of the effects from low concentrations of fluoride in water, less than that required for manifest endemic prevalence. When chronic fluorosis of water-borne origin is eliminated, a residual incidence may well remain as it has in typhoid; and again as in typhoid, attributable to sources distinct from those concerned with dramatic outbreaks.

Chronic endemic dental fluorosis is associated with water supplies

which contain 1 part per million or more of fluorides.[15,57b] The content of fluorine in a given water supply is influenced by geological conditions. Judgment should accordingly be based on the average of a number of examinations over a period of months. Supplies containing 1 to 4 parts per million are frequent in certain areas and some with as much as 20 parts per million have been reported in the United States.[24a] Mottled enamel can occur, however, when the water supply contains less than 1 part per million, although usually less than 10% of children in the community are affected and these in mild degree. Deep well and artesian waters more commonly contain fluorides in amounts dangerous to health than do surface waters, although much depends on the geological formation of the particular region.[40]

Few foods are wholly free from fluorine. The fluorine associated with plant products may be a constituent of the food itself, be admixed, or deposited on the food. That within the plant itself can be derived from the soil, the fertilizer used, or the irrigation water. External contamination comes from dusts and sprays. The great variation in conditions under which food is produced, as well as the varying capacity of plants to take up fluorine from the soil, suggests essential differences in the fluoride content of plant foods. A monograph by McClure,[33] just recently available, summarizes a series of studies on the fluorine content of foods. Values for plant foods are expressed on the basis of dry substance; animal, fish and other substances on the basis of the food as consumed. Beans, wheat, oats, rye and corn commonly contain 1 to 3 parts per million. Wheat in a certain fluorite area contained 226 parts per million. Green vegetables such as beans, broccoli, celery, cabbage, lettuce and spinach, and such fruits as apples, may have higher amounts of fluorine than the seeds, especially if the plants are sprayed. Roots and tubers, such as turnips, carrots, potatoes and peanuts, usually have less than 1 part per million.

Meat products usually contain much less fluorine than might be expected; on the basis of the fluorine content of water and plants consumed by the animals from which they are derived. The selective storage in bone spares those parts most frequently used as food. Nearly all salt water fish contains appreciable amounts, varying from 1.5 parts per million in oysters to as high as 12.5 parts per million in canned salmon. Fresh water fish contains no more fluorine than meat.

Exposure to dusts containing large amounts of fluorides is an important factor in certain parts of Africa,[28] but not in the United States. Sources of exposure in industry have little relation to mottled enamel, because of the peculiar conditions that essentially restrict development of the disease to childhood. No case of mottled enamel has been attributed to medicines or disinfecting agents, probably because they are used for relatively short periods. Nursing mothers exposed to fluorine in industry[47] have apparently transmitted the condition to their infants.

Not much is known of the epidemiologic significance of sources other than water. As independent factors in causing classical mottled enamel, they probably are not important. They may well supplement the fluorides derived from water, where the level is borderline, to such an extent as to be the determining factor. The place of fluoride containing foods will be better appreciated as more is learned about what follows

long continued ingestion of minimal and subtoxic amounts of fluorine. Probably a good deal depends on individual variations in intake and in food habits. Given comparable conditions of dosage and time, experimental evidence would indicate that foods containing fluoride are as active[33] in producing mottled enamel, as are waters.

Prevalence. An extensive study of the distribution of mottled enamel in the United States was made by Dean[14a] in 1933. From the literature, by questionnaires to dental societies and individual dentists, and by field studies, he found 97 areas where mottled enamel was definitely a problem, 98 in which it probably existed and 5 others for which data were incomplete. Later information[16] indicated about 375 known affected localities distributed through 26 states. The general area most affected in this country is the Southwest, including the states of Texas, New Mexico, and Arizona.[55] The largest concentration of affected communities is in the panhandle-west Texas region.[17] A second general region is south of the Great Lakes, from Ohio[50] to the Dakotas.[18] These three states, on the basis of present information,[40] have more affected communities than intervening jurisdictions such as Wisconsin,[22] and Illinois,[65] but the condition is well recognized in all of the area. The disease is known to occur in several of the Rocky Mountain and Pacific Coast states, and a limited focus has been determined in Virginia and the Carolinas. Of the usual groupings of states, all have been concerned except the New England and Middle Atlantic regions. The actual distribution by states and the relative frequency of the condition can only be approximated, because some state health departments have done little or no survey work.

Mottled enamel is by no means a problem confined to this country. Its world distribution is substantiated by recent reports from Canada,[62] China,[63] Japan,[59] and India[45] among others. The general world prevalence as determined by Dean,[14d] McKay,[36f] and Roholm,[47] shows that mottled enamel in the Americas occurs not only in the United States and in Canada, but also in Mexico, Argentina, Barbados and the Bahama Islands. The disease has been infrequently identified in Europe but is known to be present in Italy, Spain, England and probably Holland. In Africa the condition is widespread in Algeria, Tunisia and Morocco where there are large phosphorite deposits. It is also known in the Sinai peninsula and in certain parts of South Africa, as well as in the Azores and the Cape Verde Islands. In Asia the affection has been reported from several places in North China and Japan. Australia is the only continent where mottled enamel has not been found. As the clinical nature and epidemiologic relations of mottled enamel become better appreciated, present information will likely be found to represent but a fractional part of the actual distribution.

A naturally acquired disease of domestic animals, analogous to mottled enamel in man, was first reported by Velu[61a] in his studies of *darmous* in North Africa. Dean[14c] found a somewhat similar condition among dairy cattle in Horry County, So. Carolina. Because of these observations, the experiments of Phillips, Hart, and Bohstedt[43a] have special interest. Even when cows were fed fluorides in sufficient amounts to produce toxic symptoms, there was very slight excretion in the milk. Furthermore, storage of fluorine in muscle is slight.[33]

The cow would seem to be of little importance as an intermediate source of fluorine, either through milk or meat.

Age Incidence. Susceptibility to mottled enamel is limited to a sharply defined age group. The reason is that fluorine can be deposited in enamel only during the time that the crowns of the permanent teeth are calcifying. This period is from infancy, when the central incisors may be affected, until 16 years of age when calcification of the crowns of the last teeth, the third molars, has been completed.[20] A further characteristic of the disease is the long lag between the time when the actual damage is done and the time that it becomes objectively apparent, corresponding to the interval between completion of calcification and actual eruption of the tooth. Because the various teeth calcify at different periods, intermittent or interrupted deposition of fluoride affects only certain teeth. Dean cites illustrative experiences where older high school children were observed with all teeth normal except the third molars, these being persons who had taken up residence in the endemic area when 8 to 10 years of age. By contrast, high school students in other localities have had all permanent teeth severely affected except the third molars, which corresponded with the time a change was made to a satisfactory water supply. Such examples are unusually significant in determining the part of water supply in production of the disease, for the element of individual susceptibility is eliminated. The frequency of the disease bears no relation to color or sex[14d] nor are weather and latitude of moment.

Mottling of the enamel of temporary teeth is very uncommon. This is explained by the fact that for these teeth enamel formation is practically completed by the time of birth, and that the placenta has a protective influence.[27] The principal food of the infant, milk, contains only slight amounts of fluorine.[43a] Furthermore, the rate of development of the deciduous teeth is faster than that of the permanent teeth,[48] and it is known that the faster a tooth develops the higher is the concentration of fluorine necessary to cause mottled enamel. Under special conditions these protective factors may not be sufficient to prevent mottling.[29,53,57a]

Mottled enamel cannot develop in persons exposed after the sixteenth year of life. However, the dentin may be adversely affected, resulting in general weakening of the tooth structure.[56] The impression exists,[14d,47] unsupported by satisfactory evidence, that adults exposed to relatively large amounts of fluoride have an increased incidence of gingivitis. Teeth of adults unaffected by mottled enamel have been repeatedly shown to contain fluorine. Roholm[47] also believes that adult teeth subjected to fluorine have an increased growth of both cement and dentin, and a consequent narrowing of the pulp cavity. Other possible effects of chronic fluorosis in adults, such as changes in bone and action on the thyroid, have already been mentioned.

Methods for Field Surveys. Mottled enamel has been shown to be of sufficiently wide distribution that most individual health officers will want to know to what extent the problem is a matter of concern in their immediate jurisdictions.

The first thing to do is to look at a map. The geographic areas involved have been fairly well defined. Chemical examination of the

community water supply will demonstrate whether or not fluorides are present in sufficient amounts to constitute a potential hazard. On the basis of that information a few hours spent with dentists of the community will soon determine whether dental conditions corresponding to mottled enamel are occurring in the permanent teeth of children, and how long that situation has existed. This information will determine the need for a field survey. An adequate sample of school children is selected, of ages ranging from 6 to 16 years. Individual histories are obtained in respect to principal and secondary water supplies that have been used—how constantly and for how long. The information is confirmed by interviews with the parents. The most important part of the survey is a satisfactory dental examination by a practitioner familiar with the disease. The results of individual examinations are interpreted according to the scheme proposed by Dean, Dixon, and Cohen,[17] from which is determined the mottled enamel index. This is an arbitrary measure of the extent and severity of mottled enamel in a community. If less than 10% of the sample of children examined show mottled enamel, the index is considered negative. A negative index is sometimes mistakenly interpreted as meaning that the community is free from mottled enamel. This is not necessarily true. It simply means that the condition is not prevalent in endemic proportions. The 6 positive gradations expressed by the index, ranging from borderline to very marked, are based on a combination of numbers of persons affected—always more than 10%—and the general severity of the disease. The determination of any 1 of these 6 situations shows the need for active preventive measures.

Control of Mottled Enamel. Prevention is the only method of control, since very little can be accomplished by treatment of the disease once it has developed. In the light of much experience, McKay[36] states that cavity margins can only be established with difficulty because of the chalky texture of the teeth. Stains near the surface may be remedied by grinding off the surface of the tooth, down to normal enamel. A porcelain jacket crown is frequently applied for cosmetic reasons. When mottled enamel occurs with the average level of severity that has been noted in Arizona,[56] the cost of adequate dental care to the individual up to adulthood is about a thousand dollars, at which time the natural teeth must frequently be replaced with artificial dentures.

Considering the disfiguring effect of mottled enamel, that it is not subject to improvement by natural process or medical treatment, that every child born and reared in an endemic area is almost certain to have the condition in some degree, it is evident that chronic endemic dental fluorosis is a problem worthy of serious attention. As with most situations in preventive medicine, there is no single ideal program for control, practicable under all circumstances.

The most obvious thing to do is to get a water supply having a content of fluorides within safe limits. Depending on local conditions, sources of fluorine other than water may have to be given consideration. Attention to certain features of hygiene and of the general environment can be helpful.

With respect to the water supply, the most economical procedure in the long run, is to provide a new source of supply. The convincing

results obtained by Oakley, Idaho,[36e] Bauxite, Arkansas,[20] and Andover, So. Dakota, are sufficient to prove that this measure is completely satisfactory.[16] If the existing supply contains unusually large amounts of fluoride, it is about the only thing to do, because of the difficulties and cost of purification. The choice of the new supply depends upon local geologic conditions. In Illinois,[65] for example, surface waters are almost always low in fluorides, and can be readily substituted for supplies drawn from deep wells. Occasionally a rather sharply defined offending geologic stratum can be avoided[55] by staying above it or by going deeper and casing it off.

Providing the fluoride content of the existing supply is not excessive, an alternative procedure is to dilute the water with that from a second source essentially free from fluorides, thereby bringing the final content of fluorine below 1 part per million. The method is to be considered, when available surface supplies are alone insufficient to meet the requirements of the community. Such supplementary supplies can be obtained from shallow wells, small lakes, gravel packed wells in creek bottoms, or similar sources.

In a considerable number of small areas, and in at least one large region of the United States, the panhandle of Texas, there are few adequate fluoride-free sources of supply. The water must then be treated. This is usually an expensive procedure if all the water used in the community is processed. Treatment can be limited to that used for cooking and drinking, but with the objections that always attend dual water supplies. Of the many processes proposed,[40] the lime method[50] appears to be the most economical when it can be used. Treatment with tricalcium phosphate[5] is rapid, cheap and effective, but has not yet had sufficient field tests to fully establish its usefulness. Mechanical agitation of water with an added chemical such as magnesium oxide,[24b] and subsequent settling, has the disadvantage of being a slow process and requiring more attention than do filtration methods.

Food is the other principal consideration; just how important, remains to be determined. That some foods demand more attention than others has been demonstrated. The prohibition of fluoride sprays for vegetables and fruits is a means of protection. Fluoride compounds should not be used as a preservative of foods. The use of steam pressure cookers as a substitute for the boiling of vegetables is not known to have been previously suggested, but appears wholly logical in regions where fluoride bearing waters are common. By this method vegetables are cooked in a suspended container, and concentration of fluorides in the prepared food is avoided.

A well balanced diet will not prevent mottled enamel.[56] Avoiding a deficiency of vitamins C and D and of calcium is to be recommended, if for no other reason than to prevent the independent damage such deficiencies may cause in teeth. More milk and less water in the diets of children serves to decrease total intake of the element. In certain parts of Africa and South America protection must be afforded against dusts containing fluorides, a hazard not present in other countries.

Summary. Mottled enamel is a hypoplastic dental abnormality, caused by ingestion of fluoride compounds at any age between infancy and 16 years. The number of teeth involved depends upon the time

and duration of the exposure. The severity of mottling depends upon the amount of fluorine taken into the body during the period of calcification of the enamel. The disease primarily affects the permanent and not the deciduous teeth. Once developed, the condition is permanent. Water supplies are the principal source of the causative agent, but others, such as foods, must be considered. All of the things that give rise to mottled enamel enter into the life of people of all ages. Therefore, a program for control must be conceived on a community basis.

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PHYSIOLOGY

PROCEEDINGS OF
THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA
SESSION OF JANUARY 16, 1940.

The Study of Reproduction in Rats on Purified Diets. CLAIRE FOSTER, DORIS RUSSELL and J. H. JONES (Departments of Pediatrics and Physiological Chemistry, University of Pennsylvania, and the Children's Hospital of Philadelphia.)

A Study of Isolated Muscle Fibers From a Protoplasmic Standpoint. L. V. HEILBRUNN (Department of Zoölogy, University of Pennsylvania). The protoplasm of muscle cells resembles the protoplasm of various other types of cells in being peculiarly sensitive to the calcium ion. If an isolated frog muscle fiber is immersed in isotonic calcium chloride solution, the ends immediately become plugged. This is a reaction presumably identical with the surface precipitation reaction as studied in marine egg cells and in protozoa. As the calcium entering the ends of the fiber comes into contact with more and more of the protoplasm, the fiber becomes transformed into plug material and shortens until it is only about one-fourth of its original length.

When isolated muscle cells are placed in solutions which tend to rob them of calcium ion, they become insensitive to electric condenser discharges. Their irritability is regained on return to solutions containing calcium.

These facts are interpreted in terms of the calcium release theory of stimulation.

The Influence of Cutaneous Atmospheric Oxygen Absorption Upon the Apparent Total Oxygen Utilization of the Body. ALFRED H. CHAM-

BERS and SAMUEL GOLDSCHMIDT (Laboratory of Physiology, University of Pennsylvania). Respiratory oxygen exchange was determined by the Tissot method on subjects enclosed up to the neck in a metal box. The gaseous atmosphere surrounding the body could be changed at will.

Two of four subjects increased their oxygen consumption from the inspired atmospheric air up to 10%, or more, when moist nitrogen replaced the room air surrounding the body. In the other two subjects the rise, under similar experimental conditions, was either entirely absent, upon occasion, or, at other times, increased to a slight degree.

The results can be explained on the assumption that the oxygen for cutaneous metabolism is partially, at least, derived from the surrounding air. When deprived of the atmospheric source, an increased utilization from the blood is demonstrable. The oxygen which penetrates the skin may be used entirely by the cutaneous cells or, when its tension is high, it may gain entrance into the blood of the skin; at other times it is possible that, because of a higher tension of oxygen in cutaneous blood due to increased skin circulation, little is utilized from the atmosphere.

The Rôle of the Hypothalamus in Cardiovascular Regulation. R. F. PITTS, M. G. LARRABEE, D. W. BRONK, and F. H. LEWY (Eldridge Reeves Johnson Foundation, University of Pennsylvania). In order to evaluate the rôle of the hypothalamus in cardiovascular regulation, we have compared the character of the spontaneous activity in peripheral sympathetic nerves, as well as its reflex modification, before and after extirpation of the hypothalamus. In addition, we have studied the activity induced in these nerves by stimulation of the hypothalamus. In these latter experiments stimulating electrodes were oriented in the supra-optic and tuberal portions of the hypothalamus by means of the Horsley-Clark stereotaxic instrument, and brief repetitive condenser discharges delivered at controlled intensities and frequencies.

The characteristic grouping of spontaneous activity in the various fibers of the sympathetic nerve trunks and their reflex inhibition and excitation were in no wise altered by extirpation of the hypothalamus.

Stimulation of the hypothalamus at frequencies above 10 per second produced an increase in sympathetic activity in the inferior cardiac and cervical sympathetic nerves, and after a brief latency a rise in blood pressure. Frequencies from 1 to 10 per second often produced an inhibition of sympathetic activity and a fall in blood pressure. Records from few fiber preparations indicated that the individual units discharged more frequently and that more units were brought into activity as either stimulus frequency or intensity were increased.

It has been possible to record from single fibers of the cervical sympathetic activated from the hypothalamus. Frequency of discharge was greatest at the beginning of stimulation, but after a period of a second or so, a steady state was attained and the unit discharged rhythmically, the frequency of discharge being directly related to the intensity and frequency of hypothalamic stimulation.

There is, following stimulation of the hypothalamus, an abrupt inhibition of all spontaneous sympathetic activity and a reduced excitability of the lower sympathetic centers to either reflex excitation from afferent nerves or to hypothalamic stimulation.

These experiments show how the chemical and reflex control of sympathetic centers are modified by varying degrees of hypothalamic activity.

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